Mineral Management in African Indigenous Vegetable Production Systems

DISSERTATION

For obtaining the academic degree
Doctor of Philosophy (Ph.D.)

Submitted to the Faculty of Life Sciences of the Humboldt-Universität zu Berlin

By **Godfrey Nakitare Nambafu**

President of Humboldt-University of Berlin: Prof. Dr.-Ing. Dr. Kunst

Dean of the Faculty of Life Sciences: Prof. Dr. Grimm

Doctoral Degrees Committee
1. Prof. Dr. Christof Engels
2. Prof. Dr. Andreas Bürkert

Date of disputation: 06.07.2018

Acknowledgements

I would like to gratefully acknowledge the guidance and supervision of Prof. Dr. Christof Engels. It is through his corrections, criticism and guidance that I was able to write this scientifically well formulated document. I am also indebted to Dr. Joseph Patrick Onyango Gweyi, for his advice towards my PhD work and not forgetting Holger Besser and Annika Nerlich for their mentorship during my stay in Berlin.

Deserving of special mention is HORTINLEA (Horticultural Innovation and Learning for Improved Nutrition and Livelihood in East Africa) research project, for their financial support and skills accorded throughout my study period in Germany and Kenya.

I am also thankful to my late dad, Abel Nambafu and mum, Scholastica Namalwa for their spiritual support and motivation. Not to forget my wife, Naomi Kollongei and my two daughters Melony Sipha and Ruby Zawadi for their prayers, moral support and encouragement.

Table of Contents

List of tables

List of figures

List of abbreviations

AIVs – African Indigenous Vegetables

Al – Aluminum

ANOVA – Analysis of Variance

ASDSP – Agricultural Sector Development Support Programme

AVRDC – World Vegetable Center

Ca – Calcium

Cd – Cadmium

CODEX – Codex Alimentarius collection of food standards

Cu – Copper

DAP – Days after planting

DAS – Days after sowing

DMRT – Duncan Multiple Range Test

EFSA – European Food Safety Authority

FAO – The Food and Agriculture Organization of the United Nations

Fe – Iron

$FePO_4$ – Iron phosphate

Fig. – Figure

g – Grams

GDP – Gross domestic product

GOK – Government of Kenya

Ha – Hectares

HNO_3 – Nitric acid

ICP – OES – Inductively Coupled Plasm with optical Emission Spectroscopy

IPUE – Internal Phosphorus Use Efficiency

JECFA – Joint Expert Committee on Food Additives

K – Potassium

Kg – Kilograms

KH_2PO_4 – Potassium phosphate

KNBS – Kenya National Bureau of Statistics

m – Meters

Mg – Magnesium

Ml – Milliliter

mm – Millimeters

Mn – Manganese

N – Nitrogen

P – Phosphorus

Pb – Lead

Rock P / $CaPO_4$ – Rock Phosphate fertilizer

RSA – Research Solution Africa

S – Sulphur

sSA – Sub-Saharan Africa

Ton – Tonnes

TP – Transplanting

UNESCO – United Nations Educational, Scientific and Cultural Organization

WHO – World Health Organization

Zn – Zinc

Abstract

African Indigenous Vegetables (AIVs) have recently captured considerable attention as "super vegetables" due to their nutritional and environmental benefits (Cernansky 2015). As AIVs include many species belonging to different botanical families, more species-specific knowledge e.g. on nutritional value and agronomic management is needed to fully exploit those benefits. In this thesis we compared leafy AIV species from five families including C_3 and C_4 species, and a legume and non-legume species (*Brassica carinata*/Cruciferae - Ethiopian kale, *Amaranthus cruentus*/Amaranthaceae - amaranth, *Vigna unguiculata*/Leguminosae cowpea, *Solanum scabrum*/Solanaceae - African nightshade, *Cleome gynandra*/Capparaceae - spider plant) with a non-indigenous species, which is commonly grown in Kenya (*Brassica oleracea acephala/*Cruciferae - "exotic kale"). We compared the leaf concentrations of beneficial nutrients and heavy metals as an index for the nutritional value, the performance under different rates and forms of phosphorus (P) supply as an index for adaptation to suboptimal chemical soil conditions, and the harvest-related nutrient outflow from soil as an index for fertilizer need. The thesis was embedded in the interdisciplinary research program HORTINLEA (Horticultural Innovation and Learning for Improved Nutrition and Livelihood in East Africa).

Keywords: African Indigenous Vegetables, nutritional value**,** Phosphate availability, nutrient export

Abstract (German)

Durch ihre Umwelt- und Nährwertvorteile erweckten Afrikanische indigene Blattgemüse – „African Indigenous Vegetables“ (AIV) in letzter Zeit Aufmerksamkeit als „Supergemüse“ (Cernansky 2015). Da mehrere Spezies aus unterschiedlichen botanischen Familien innerhalb der AIVs vertreten sind, ist ein besseres Verständnis Spezies spezifischer Eigenschaften, wie z.B. Ernährungswert und agronomischem Management nötig, um diese Vorteile voll ausschöpfen zu können. In dieser Arbeit haben wir Afrikanisch indigene Blattgemüsearten aus fünf Familien, inklusive C_3 and C_4 Spezies und Leguminosen und nicht-Leguminosen (*Brassica carinata*/Cruciferae - Ethiopian Kale, *Amaranthus cruentus*/Amaranthaceae - Amaranth, *Vigna unguiculata*/Leguminosae - Cowpea, *Solanum scabrum*/Solanaceae - African Nightshade, *Cleome gynandra*/Capparaceae - Spiderplant) mit einer importierten und in Kenia häufig angebauten Gemüseart (*Brassica oleracea acephala/*Cruciferae - Exotic Kale) verglichen. Wir betrachteten die Pflanzenarten hinsichtlich ihrer Konzentrationen an wertgebenden und toxischen Elementen in Blättern als Index für den Nährwert, ihrer Reaktionen auf unterschiedliche zur Verfügung gestellten Mengen und Formen an Phosphor (P) als Index für die Adaptation an suboptimale chemische Bodenbedingungen und ihre erntebedingten Nährstoffausfuhren aus dem Boden als Index für den Düngerbedarf. Die Arbeit war in das interdisziplinäre Forschungsprogramm HORTINLEA (Horticultural Innovation and Learning for Improved Nutrition and Livelihood in East Africa) eingebunden.

Keywords: African Indigenous Vegetables, Ernährungswert**,** Phosphatverfügbarkeit, Nährstoffausfuhr

Chapter 1: General Introduction

1.1 Food production and soil fertility status

Sub-Saharan African (sSA) is one of the regions in the world where people depend entirely on agriculture for their livelihood. Agriculture has largely contributed to sSA's Gross Domestic Product (GDP) but food production in this region still lags behind. The region is one of the poorest, whose per capita income in 2010 stood at \$688 compared to \$1717 in other developing countries (Chauvin et al. 2012). With a rapidly growing population in sSA, nearly one third of the population is food insecure leading to cereal imports increasing to about 25% by early 2000s (De Graaff et al. 2011).

Food production in sSA is negatively affected by land degradation with high levels of soil mining and very low fertilizer application in agriculture (Stoorvogel and Smaling 1990; Hengl et al. 2017). Low soil fertility has an impact on vegetable and grain crop production in this region, hence vegetable availability is far below 150 kg per capita per year (Ganry 2009). Currently, the soil nutrient balance in sSA, both at regional and national levels, shows a general deficit of soil minerals, especially phosphorus, in cropland soils (MacDonald et al. 2011) alongside other nutrients like nitrogen and potassium. The average mineral loss was estimated to be 660 kg N, 75 kg P, and 450 kg K ha^{-1} $year^{-1}$ in 0.2 billion hectares of cultivated land in 37 countries of Africa (Stoorvogel and Smaling 1990; Sanchez et al. 1997). Losses of up to 130 kg N, 5 kg P and 25 kg K ha^{-1} $year^{-1}$ were reported in East Africa (Smaling et al. 1996).

Different soil properties have led to nutrient gradients in soils at the county level, sub-county level and villages. Additionally the socio-economic status of the farmers (wealth) influences the farming systems used and thereby nutrient gradients within farms or even individual field levels (Haileslassie et al. 2005). Other factors include the previous and current ground/ field cover, which is related to the crop species and production strategies employed in the management of the plants and the soil. Most of the soil nutrients are lost through harvested crop organs and crop residues, which can be quantified with modeling (Faerge and Magid 2004). These harvested organs are mostly the basis of human nutrition. Nevertheless, it should be noted that these nutrients were obtained from the soil and need to be replaced for sustainable crop production.

Soil nutrients like P, may also be limited or unavailable due to acidic nature of some soils in sSA, phosphate is bound in Fe- and Al-phosphates, which reduce P availability for plants. For this case, production of vegetables with high uptake and internal P utilization may be advantageous. Plant species exist, which developed different adaptive traits for P deficiency, which might mobilize P out of adsorbed P fractions (Schnug and Haneklaus 2016). The use of such plants may reduce the application of P fertilizer and thus cut down the cost of food production and reduce environmental damages as a result of eutrophication.

1.2 Human health, nutrition and risks associated with crop production

Human health is associated with food. Food should provide the required minerals and vitamins that the body needs to perform effectively. Due to poverty and suboptimal production of food, the majority of people, especially children, are malnourished in sSA, Malnutrition includes micronutrient deficiencies, under-nutrition and hunger (Fanzo 2012). Lack of vitamin A and minerals like Ca, Mg, Fe and Zn are widespread among people in this region. Africa records for about 10 % of wasting, 25 % of underweight and 39 % of stunted growth in children aged below five years with sSA accounting for one third of all undernourished children globally, by 2015 (Akombi et al. 2017). This problem might be solved by promoting the consumption of vegetables rich in nutrients, which are found in sSA by all individuals despite the economic or social class. Consumption of vegetables in sSA has been declining over the past years due to preference for starchy plant products that quickly satisfy hunger and provide required energy (Chauvin et al. 2012). African indigenous vegetables (AIVs) are known to contain higher levels of vitamins, minerals and have medicinal properties (Makokha and Ombwara, 2005). Until now AIVs have been under-utilized and yet they can be the preferred remedy to address the mentioned health problems.

In addition, heavy metal contamination is also rampant in agricultural crops, especially in urban and peri-urban areas. Heavy metals, e.g. cadmium and lead are of environmental concern and continuous uptake of these metals within human diet is detrimental to human health. Diseases associated with heavy metals include hemoglobin synthesis disorders, nervous system diseases, cancer and kidney disorders (Okoronkwo et al. 2005; White 2012). Crops growing in the farm are contaminated with heavy metals either through the systemic pathway by uptake of heavy metals from soil solution by the roots or by adhering contaminated soil or dust particles from atmosphere or contaminated water for irrigation (Muchuweti et al. 2005; Kapungwe 2013; Kananke et al. 2014). Many leafy vegetables, including AIVs, are super accumulators of anti-nutritional components like heavy metals (Shackleton et al. 2009). Translocation and accumulation of these heavy metals to harvested or edible plant parts depends on the plant genotype, climatic factors, soil characteristics and agronomic management (McLaughlin et al 1999). Comparing the concentration of heavy metals in vegetables with world health organization (WHO) maximal dietary weekly intake limits of heavy metals is credible to avoid diseases.

1.3 African Indigenous Vegetables in Sub Saharan Africa

AIVs are leafy, domesticated vegetable species, that were in the past treated as 'wild' plants, which make part of the diet in the rural, poor communities in Africa (Shackleton et al. 2009). Sub-Saharan Africa is a renowned natural habitat for over 40,000 AIV species, out of these, 1,000 can be consumed as vegetables (Muhanji et al. 2011). In East Africa, the most consumed ones are amaranth (*Amaranthus* sp), pumpkin leaves (*Cucurbita* sp), jute plant *(Corchorus molitorius*), sunnhemp *(Crotalaria brevidens*), cowpea (*Vigna* sp), black nightshade *(Solanum nigrum*), spider plant (*Gynandropsis gynandra*) and Ethiopian kale (*Brassica carinata*) among many others.

Over long time, these vegetables have been associated with poverty and that's why the rich and middle class people rarely consumed them. Due to neglect, the production and distribution of this vegetables across countries is too low (Habwe et al. 2008). With the increase of diseases and malnutrition, indigenous vegetables are increasingly becoming valued in sSA countries, among people of all cultures, traditions and religions (Smith and Eyzaguirre 2007). Thus, the growth, distribution and utilization of these vegetables have to be enhanced through research and innovation.

1.4 Growth of AIVs in Kenya and their importance

In Kenya, horticulture is among the leading contributors to the GDP accounting for 36% (vegetables 36%, flowers 30%, fruits 26%, nuts 5% of domestic value) and 38% of export earnings and continues to grow between 15 and 20% per year (HCD 2016). AIVs are among major vegetables produced in Kenya, alongside tomatoes (*Solanum lycopersicum*), cabbages (*Brassica oleracea var. capitate*), snow peas (*Pisum sativum var. saccharatum*), kales (*Brassica oleracea acephala)*, spinach (*Spinacia oleracea*), runner beans (*Phaseolus coccineus*), French beans (*Phaseolus vulgaris*), carrots (*Daucus carota*), broccoli (*Brassica oleracea var. italic*) and Asian vegetables (HCD 2016).

Indigenous vegetables have contributed immensely to human diet in Kenya although a large population preferred exotic vegetables like spinach, cabbage and exotic kales. The vegetables are majorly grown in Kisii, Kakamega, Nakuru and Kiambu County. Recently, most people are purchasing traditional vegetables for consumption (Ngugi et al. 2007). The demand is also high in Kenya's bigger and smaller towns as well as in Uganda and Tanzania (Weinberger and Msuya 2004). Nevertheless, communities are still selective in vegetables they eat based on their culture and availability. High consumption has led to high demand of AIVs (Shiundu and Oniang'o, 2007; Habwe, 2008).

The vegetables are able to tolerate diseases, harsh climatic conditions and easily adapt to local atmospheric conditions, hence can be grown throughout the seasons. The growing of vegetables can be a better source of income and employment especial in rural areas (Muhanji et al. 2011). The raising status of AIVs will highly contribute to alleviation of food insecurity, malnutrition and poverty in Kenya and other countries in sub-Saharan Africa.

1.5 Interest in AIVs

Enhancement of soil fertility levels results in high vegetable yield on poor soils. Most farmers add organic and inorganic fertilizers to replenish their soils nutrients, but the amounts are far below the recommended ones. At some point the recommended levels are not species-specific (Shackleton et al. 2009), since species differ in amount and form of nutrients required. Some species might use different nutrient forms more efficiently than other species, e.g. P in form of rock P or Fe-P or are able to tolerate deficiency and yield more than the other species. Regarding AIVs, there is less information regarding their ability to perform under different forms of essential nutrients and their ability to perform well under nutrient deficiency. Furthermore, there is no information on fertilizer recommendations that match the amount of nutrients taken out through harvesting of these species. Since, agronomic practices like harvesting techniques and production systems differ among farmers, they were used in our study to generate information that can be important to policy makers, extension officers and farmers. It would be important to identify:

i) AIV species with highest P use efficiency

ii) AIV species removing less minerals from the soils although producing sufficient edible biomass and

iii) Which production or harvesting system removes fewer minerals from soil.

Most people in sSA consider AIVs to be of less value since they have no information on their nutritional value (Kamga et al. 2013). People regard AIVs as poor or low status food for people who cannot afford 'proper' (exotic) vegetables and fruits. Shackleton et al. (2009), states that AIVs are vital food components of human diets and they possess essential nutritional value for the development and better health of the human body. The amount of distinct nutrients present in each species tested will act as a basis for nutritionist to be able to adequately instruct people (patients) on the number of times a week a patient has to eat a particular vegetable(s) in order to get required nutrients. There has been a general notion that AIVs are sources of essential minerals, but less information is available on which species is best suited in addressing certain deficiency or health problems.

Improper packaging, transportation and poor handling of AIVs at the market place may result in heavy metal contamination. In the urban and peri-urban areas, pollutants, which may contain heavy metals from industrial, commercial, residential and other urban activities are of environmental concern and may be found in the vegetables. It was important to know, which AIVs accumulate less heavy metals. There was need to distinguish between the contaminants adhering on particulates from the atmosphere and those that are taken up by the AIVs during growth to advice consumers and traders on proper way of handling vegetables before consumption and farmers for consideration of planting site and species before planting vegetables.

1.6 Aims

Our study was an integral part of the HORTINLEA (Horticultural Innovation and Learning for Improved Nutrition and Livelihood in East Africa) research project. HORTINLEA is an interdisciplinary research program with the purpose of addressing food security in East Africa, particularly in Kenya and Tanzania. The main aim of the program was to improve peoples' livelihood and nutritional situation among rural and urban poor. In order to address these problems, HORTINLEA used an integrated approach that targeted the entire food chain (from production to consumption) of AIVs. This resulted in the formation of 14 sub-projects whereby our research work was part of the first three subprojects that addressed problems in production of AIVs. Our subproject (subproject 3) looked at soil related issues affecting production with a main focus on five AIVs namely *Solanum scabrum*, *Amaranthus cruentus*, *Vigna unguiculata, Cleome gynandra* and *Brassica carinata.* An exotic vegetable, *Brassica oleracea acephala*, species was also included in the study for comparison purpose.

The main objective of subproject 3 was to provide knowledge and information on species-specific nutrient requirements and vegetable nutrient composition with regard to essential and heavy metals. The specific objectives addressed in this study are:

- To assess phosphorus use strategies of leafy vegetables: Responses of species to different rates and forms of phosphorus supply
- To quantify nutrient fluxes in AIVs: Effects of species, harvesting technique and production system on nutrient export from soil
- To assess nutritional value of AIVs: Essential and heavy metal concentration from open air and supermarkets in Nairobi

These objectives will be illustrated in chapter 2, 3 and 4 in more detail.

Chapter 2: Nutritional value of AIVs

This chapter was divided into two sections; the first section is dealing with essential macro and micro elements, and the second section with heavy metals.

2.1 Nutritional value of AIVs: Essential element concentrations of samples from open air and supermarkets in Nairobi

2.1.1 Abstract

Deficiency of macro elements such as calcium (Ca) and micro elements like zinc (Zn) is widespread in sub-Saharan Africa, particularly among the poor who strongly depend on staple crops like maize and sorghum for food. Increasing diet diversity through utilization of AIVs might be an option to reduce hidden hunger caused by macro and micro element deficiency. In this study, essential elements were measured in edible plant organs of vegetables sold on markets in Nairobi to assess potential health benefits associated with consumption of AIV. Fresh samples of AIVs; African nightshade (*Solanum scabrum)*, amaranth (*Amaranthus cruentus)*, cowpea (*Vigna unguiculata)*, spider plant (*Cleome gynandra)*, Ethiopian kale (*Brassica carinata*) and a standard species commonly grown in Kenya, exotic kale (*Brassica oleracea acephala* group) were collected from ten open air and five supermarkets. Macro (potassium - K, phosphorus - P, magnesium - Mg, sulphur - S and calcium - Ca) and micro (iron - Fe, zinc - Zn, copper - Cu and manganese - Mn) elements were measured.

The results showed that macro and micro element concentrations were similar in vegetable species irrespective of the market type (open air or supermarkets). Element concentrations in most vegetable species showed no differences between seasons, apart from K that showed much higher concentration in the wet season than the dry season in all the species. Exotic kale, Ethiopian kale and African nightshade had higher Fe concentrations in the wet season. Vegetable species differed in the amount of element concentrations with amaranth having the highest concentrations of K, Mg, Ca, Fe and Zn. Spider plant was higher in Ca, P and Cu, and cowpea higher in Mn, while exotic kale showed higher concentrations of S and Ca. Among the AIVs, cowpea was the species with the lowest concentration of most minerals including K, Mg, P, S and Zn, whereas exotic kale was lower in most element concentrations compared to AIVs.

Keywords: Hidden hunger, nutritional value, essential elements, external contamination

2.1.2 Introduction

2.1.2.1 Food security and nutrition

Nutrition is a broader term that means intake of food in relation to the body needs. Nutrients needed are divided in to two broader groups namely macro nutrients, which include carbohydrates, lipids and proteins, and micro nutrients (Mahan and Escott-Stump 2000). Minerals that are needed by the body in large amounts are macro elements, while micro elements are needed in trace amounts (FAO 2004a). Low dietary micro nutrient supply is leading to malnutrition, which is commonly referred to as hidden hunger. Hidden hunger is a major food security problem affecting millions of people across the world. Out of the 20 most important human health risks/ problems world-wide, three are a result of macro and micro element malnutrition (Stein 2010).

Malnutrition has become a global issue with under nutrition leading to death of millions of children yearly (Ismail and Suffla 2013). Sub-Saharan Africa is among the regions, where deficiency of macro elements such as calcium (Ca) and micro elements like zinc (Zn) is wide spread (Caulfield et al. 2006). In Kenya, half of the population is unable to meet their nutritional demands. AIVs are likely to provide the minerals/elements to counteract deficiencies affecting millions of the population. In most African countries, Kenya included, concentration has been focused on improving and increasing mechanisms for food availability through innovative technologies with less concern on nutritional and health aspects, thus, high quality nutritious food is now a challenge in Africa (Fanzo 2012). In Africa continent, about 40% of children under 2 years are persistently malnourished. This is not associated with the quantity of food they are fed, but rather poor quality food. In a year, 10.8 million child deaths occur globally, of which 19% of the deaths are associated with Fe, Zn, iodine and vitamin A deficiencies. Generally, in developing countries, 54% of deaths in children is associated with malnutrition (Bain et al. 2013). In addition, 2.2 billion people, particularly pregnant mothers and children suffer from iron related anemia (Hart et al. 2005).

The consumption of indigenous species is low for the fact that most households are unable to access them (Ekesa et al. 2009) due to low market availability. A study by RSA (2015), found out that the most consumed leafy vegetables in Kenya's big cities (Nairobi, Mombasa and Nakuru) were exotic kale (42%), spinach (42%), cabbage (29%), leaf amaranth (16%), African nightshade (12%), cowpea leaves (7%), whereas, Ekesa et al (2009), found cowpea leaves (85%) followed by jute mallow (63.3%) to be the most consumed vegetables in rural areas of western Kenya. The problem of malnutrition can be addressed through eating food rich in nutrient elements like Mg and Fe. Although AIVs are said to be rich in macro and micro elements like Ca, Mg, Fe and Zn, little information concerning their concentrations and bioavailability exists.

2.1.2.2 Essential elements in AIVs and their importance in addressing hidden hunger

Vegetables form part of our daily food intake with dietary recommendation of 400g per day per person (FAO 2005). Vegetables are known to be the reliable and accessible source of energy based on their wide and easy availability. There are two groups of vegetables in sSA i.e. exotic and indigenous vegetables. Exotic vegetables have outweighed traditional vegetables in production and consumption in most countries in sSA. However, production and consumption of AIVs in Kenya is increasing yearly (Habwe et al. 2008), since they are rich in essential elements (Fe, Ca and zinc Zn), vitamins and they are highly medicinal effective (Shackleton et al. 2009). They are also rich in antioxidants, e.g. ß-carotene, (Abukutsa 2010).

In human body, Fe is present in hemoglobin and is essential in the transportation of oxygen from the lungs to the tissues. Its deficiency results in anemia. It is also a component of enzyme systems. Zn is a co-factor for many enzymes that are responsible for tissue differentiation and cellular growth. Its deficiency leads to stunted growth in children. Ca being the most abundant element in the human body, helps in metabolic processes like muscle contraction, cell adhesion, blood clotting, hormone and neurotransmitter release, skeletal strength maintenance among other roles. Its deficiency leads to week bones and bleeding disorders (Allen et al. 2006). Mg is a cofactor in over 300 enzyme systems that regulate protein synthesis, diverse biochemical reactions, blood glucose control and pressure regulation, muscles and nerve functions. Low Mg in the body has been associated with chronic illness including coronary heart disease, osteoporosis, diabetes and hypertension (Swaminathan, 2003). There are other essential elements, which are important to human health although they are not deficient in the human body. These elements are K, P, S and Cu whereby K is important in heart beat regulation and nerve conduction. Phosphorus provides energy to the body, is mostly found in bones and teeth, and helps in body protein building, while copper plays a vital role of incorporating iron in hemoglobin (Soetan et al. 2010).

To improve nutritional status of the people in sub-Saharan Africa, focus should be put on the growth and high utilization of AIVs, among other strategies. The vegetables had been neglected in the past, but considering their health aspects they could be a good remedy for malnutrition problems in Africa.

2.1.2.3 Aims

In this study, we quantified the concentrations of essential macro- and micro elements in leafy vegetables sold on markets in Nairobi with the aim to inform consumers and nutritionists about the value of AIVs for meeting human element needs. Specifically, we were interested if element concentrations are higher in AIVs than in "exotic kale" (*Brassica oleracea acephala*, common name in Nairobi, *Sukuma wiki*).

We compared element concentrations of samples from supermarkets and open air markets. It was assumed that there is quality control of vegetables sold in supermarkets not only with respect to freshness and external quality, but also concerning field management. For example, vegetables sold in supermarkets might have been mainly produced on fields supplied with mineral fertilizers. In Kenya, mineral P-fertilizer is available in the form of super phosphates, which contains Ca, P and S. Thus, we expected vegetables from supermarkets to contain higher concentrations of Ca, P and S than vegetables from open air markets.

We collected vegetable samples from all 15 markets twice, in November 2014 and in June 2015. November-sampling falls in the dry, and June-sampling in the rainy season. We wanted to know if element concentrations systematically vary with season. The availability of plant elements in the soil is higher in wet than in dry soil, because element diffusion towards the roots is increased (Kuchenbuch et al. 1986; Marschner, 1995). Thus, high element concentrations in vegetables produced in the wet as compared to the dry season, were expected.

2.1.3 Materials and methods

2.1.3.1 Study area

The study was done in Nairobi County, a county hosting the capital city of Kenya. The county is located within 1°18'S and 36°45'E and covers an area of approximately 694.9 km^2 with a population of over 3 million people (KNBS, Kenya census 2010). The county has an altitude of about 1798m (5899 ft.) and varying temperatures ranging from 12°C to 27°C annually. It experiences a bimodal precipitation with an average annual rain fall ranging from 800 – 1,050 mm. The long rains are received in March and April while short rainy season comes between November and December (JICA). This climate favours the growth of crops such as vegetables, bananas (*Musaceae sp.*), irish potatoes (*Solanum tuberosum*), maize (*Zea mays*) and coffee (*Coffea sp.),* that cover 9% of the area, (ASDSP 2015).

2.1.3.2 Sampling size

Big retail markets, selling vegetables to consumers and frequented by many buyers and sellers were selected. The selected markets were found to be evenly located within the county, with some being in the outskirts, within the city, along the road side and near residential areas. Ten open air markets (Wakulima, Kangemi, Kawangware, Kibera, Mtindwa, Kayole, Ruai, Dandora, Githurai 45 and Ruiru) and five supermarkets (Uchumi, Naivas, Nakumatt, Ukwala and Tuskys) were selected for the study. The samples collected in the markets and in the two seasons are shown in Table 2.1.

Table 2.1: Number of samples of vegetable species collected in open air and supermarkets during the dry and wet season; where ∑ denote the total number of the samples; XK – exotic kale, EK – Ethiopian kale, AN - African nightshade, AM - amaranth, CP - cowpea and SP - spider plant.

Species	Dry season		Wet season		∑		∑
	Open air market	Super-market	Open air market	Super-market	Open air market	Super-market	Total
XK	10	5	9	5	19	10	29
EK	6	4	9	2	15	6	21
NS	9	5	10	4	19	9	28
AM	10	4	10	5	20	9	29
CP	8	4	9	3	17	7	24
SP	8	1	5	1	13	2	15
∑	51	23	52	20	103	43	146

2.1.3.3 Collection of fresh vegetable samples

Plant samples were collected in two periods, in the dry spell and during the rainy season. The first sampling was carried out from 13th – 20th November, 2014, and the second sampling from 15th – 22nd June, 2015. Both samplings were done during the day, between 10 am and 6 pm. Five AIV species and one exotic vegetable species were collected. These species were African Night Shade (Managu) - *Solanum scabrum*, amaranth (Terere) - *Amaranthus cruentus*, cowpea (Kunde) - *Vigna unguiculata*, spider plant (Saga) - *Cleome gynandra*, Ethiopian kale (Kansira) - *Brassica carinata*) and exotic kale (Sukuma wiki)- *Brassica oleracea acephala*. Not all vegetables were found in all markets Table 2.2). A vendor was randomly selected from the market and about 2 kg of fresh vegetables belonging to each species bought. The samples were packed carefully in big paper bags, placed in a cooler box, and then taken to the cold chamber (10^{o}C).

Table 2.2: Collected vegetable species in open air and supermarkets during the dry and wet season; where XK – exotic kale, EK – Ethiopian kale, AN - African nightshade, AM - amaranth, CP - cowpea and SP - spider plant (open air market =10 and supermarkets = 5).

Market type	Market name	Species collected	
		Dry season	Wet season
Open air market	Wakulima	XK, AM, CP	AM, NS, CP, EK, XK, SP
Open air market	Ruai	XK, EK, NS, AM, SP	AM, NS, EK, XK,
Open air market	Kawangware	XK, AM, CP, SP	XK, EK, NS, AM, CP
Open air market	Mutindwa	XK, NS, AM, CP, SP	XK, EK, NS, AM, CP, SP
Open air market	Githurai	XK, EK, NS, AM, CP, SP	XK, EK, NS, AM, CP
Open air market	Ruiru	XK, EK, NS, AM, CP, SP	XK, EK, NS, AM, CP, SP
Open air market	Kibera	XK, EK, NS, AM, CP	AM, NS, CP, EK, XK
Open air market	Kangemi	XK, EK, NS, AM, SP	XK, EK, NS, AM, CP, SP
Open air market	Kayole	XK, EK, NS, AM, SP	XK, NS, AM, CP,
Open air market	Dandora	XK, NS, AM, CP, SP	EK, NS, AM, CP, SP
Supermarket	Nakumatt	XK, EK, NS, CP	XK, EK, NS, AM, CP,
Supermarket	Uchumi	XK, AM, CP	XK, EK, NS, AM, SP
Supermarket	Naivas	XK, EK, NS, AM, SP	XK, NS, AM
Supermarket	Tuskys	XK, EK, NS, AM, CP	XK, NS, AM, CP,
Supermarket	Ukwala	XK, EK. NS, AM, CP	XK, NS, AM, CP

2.1.3.4 Pretreatment and washing of fresh vegetable samples

All samples collected from the markets were divided into edible parts including leaves, petioles and stipules, and non-edible parts, mainly stems. The non-edible parts were discarded and the edible parts were placed in labeled bags. The samples were later divided into two bunches; one part was washed thoroughly with tap water for 2 minutes and rinsed with distilled water, while the other part was not washed at all. Then, all samples were dried in the oven at 70^{o}C until a constant weight was obtained. Dried samples were finely ground into powder form, sieved by 2 mm gauge sieves and stored in clean polyethylene bags for chemical analysis.

2.1.3.5 Sample digestion and laboratory analysis

A 250 mg aliquot of each powdered sample was weighed into a glass bottle and burned in a furnace at 500° C for 12 hours. The samples were cooled and 2.5 ml of nitric acid (21.5 % HNO_3) was added. To evaporate the acid samples were placed on a heating plate in a hood. The samples were later cooled and the residue dissolved in 2.5 ml of hydrochloric acid (12.0% HCl). The mixture was then transferred to 25 ml flask and double distilled water added up to the mark. The liquid was then filtered into a clean, well labeled plastic bottle using size 42 filter paper. The filtered liquid was subjected to inductively coupled plasma with optical emission spectroscopy (ICP – OES) analyzer for macro, trace and ultra-trace element determination in the vegetable samples. The elements analysed included, K, P, S, Mg, Ca, Fe, Zn, Cu, Mn, Cd and Pb.

2.1.3.6 Statistical data analysis

R statistical software was used to generate boxplots, means and standard deviation determination. The residuals were tested for normality using q-q plots, histogram and density mean. Homogeneity of variances was tested using Bartlett's test for equal variance. One and two way analysis of variance (ANOVA) was used to find out the significant differences ($p < 0.05$) among the plant species and interactions between species, market type and seasons. A post hoc LSD test for mean separation was also done. Unpaired T-test was used to determine significant differences ($p < 0.05$) in vegetable species between the two markets and two seasons.

2.1.4 Results and discussion

2.1.4.1 Differences among species in concentration of essential elements

For assessment of differences among species in concentration of essential elements, data from the washed samples were analysed. Chapter 2.1.4.1 contains data showing the overall variance of species-specific mineral element concentration (box plots), and data on mean species-specific mineral element concentration across the two market types and growing seasons. The median in the box plots showed how samples were distributed to the top and bottom of box plot whereas the mean denoted the average representative of samples for further use in discussion part. Chapter 2.1.4.1 was further subdivided to present and discuss data on macro elements absorbed by plants in the form of cations, i.e. K^+, Mg^{2+} and Ca^{2+} (2.1.4.1.1), and in the form of anions ($H_2PO_4^-$ or HPO_4^{2-} and SO_4^{2-}) (2.1.4.1.2), and data on micro elements (2.1.4.1.3).

2.1.4.1.1 Differences among species in the concentration of macro elements absorbed by plants as cations

Concentrations of K in edible plant organs of the vegetables varied from a minimum of 22 to a maximum of 65 g kg^{-1} dry mass (Fig. 2.1a). The range of concentrations, which covers 50% of all data, which is indicated by the extension of the boxes, was largest for African nightshade, and smallest for exotic kale. The means were 36 g K kg^{-1} leaf dry mass for exotic kale, 42 g K kg^{-1} leaf dry mass for Ethiopian kale, 46 g K kg^{-1} leaf dry mass for African nightshade, 48 g K kg^{-1} leaf dry mass for amaranth, 32 g Mg kg^{-1} leaf dry mass for cowpea and 35 g K kg^{-1} leaf dry mass for spider plant. The mean K concentrations significantly differed among species from 48 g K kg^{-1} leaf dry mass for amaranth to 32 g K kg^{-1} leaf dry mass in cowpea (Table 2.3). The mean K concentration of exotic kale, although higher, did not significantly differ from the mean concentrations of cowpea and spider plant, but was significantly lower than the mean concentrations of Ethiopian kale, African nightshade and amaranth. Data concerning K concentrations in AIVs from literature strongly vary within and among species (Adam 2016, non-published study project, section of Plant Nutrition and Fertilization, Humboldt University Berlin). For example, for spider plant, Abugre et al. (2011) indicate K concentrations in edible plant organs of 5-6 g K kg^{-1} dry mass, whereas Van Jaarsveld et al. (2014) indicate 27 g K kg^{-1} dry mass, and Wehmeyer (1986) indicate 34 g K kg^{-1} dry mass. For leafy vegetables, the sufficiency range, which indicates leaf K concentrations sufficient for supporting normal growth, is between 30 and 70 mg K kg^{-1} dry mass (Bergmann et al. 1976). Neglecting literature data on K concentrations, which would clearly indicate K deficiency (Abugre et al. 2011), our data are in the same range as data from literature (Adam, 2016).

Concentrations of Mg in edible organs of the vegetables ranged from a minimum of 2.5 to a maximum of 19 g kg^{-1} dry mass (Fig. 2.1b). The range of Mg concentrations, which covered 50% of all data, was generally smaller than the same range of K concentrations (compare the extension of the boxes in Fig. 2.1b and Fig. 2.1a). This range of Mg concentrations was largest for amaranth and spider plant, and smallest for exotic kale.

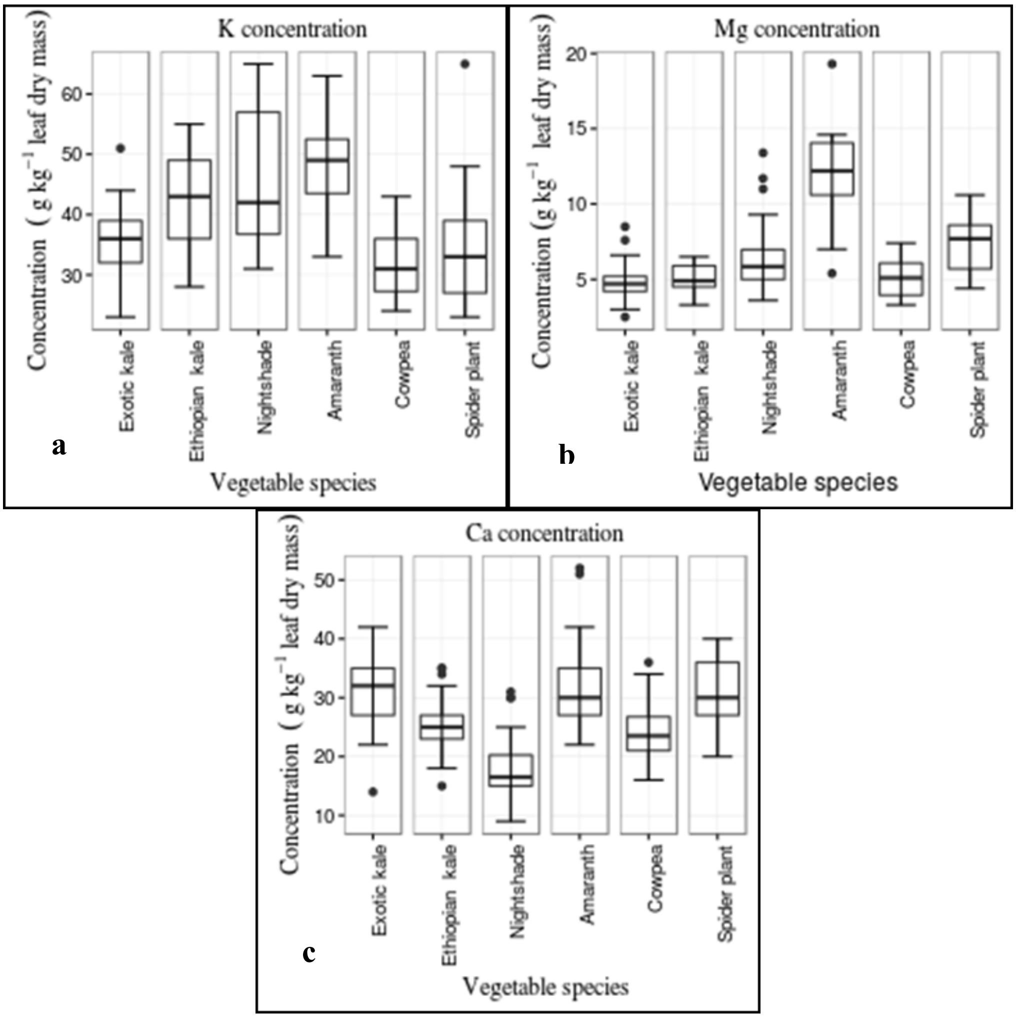

Figure 2.1: Box plots showing, minimum, first quartile, median, third quartile and maximum potassium (a), magnesium (b) and calcium (c) concentration levels (g kg^{-1} leaf dry mass) of samples from respective vegetable species. The dots represents (outliers) samples with extreme ranges from the rest, determined by $Q_3 + 3*IQR$ or $Q_1 - 3*IQR$ formula; where, Q_1 – first quartile, Q_3 – third quartile, IQR – interquartile range.

The means were 4.8 g Mg kg^{-1} leaf dry mass for exotic kale, 5.1 g Mg kg^{-1} leaf dry mass for Ethiopian kale, 6.6 g Mg kg^{-1} leaf dry mass for African nightshade, 12 g Mg kg^{-1} leaf dry mass for amaranth, 5.1 g Mg kg^{-1} leaf dry mass for cowpea and 7.4 g Mg kg^{-1} leaf dry mass for spider plant. The mean Mg concentrations significantly differed among species (Table 2.3). For amaranth, the mean concentration was more than two fold higher than for exotic kale. The mean Mg concentration of exotic kale did not significantly differ from that of Ethiopian kale and cowpea, but was significantly lower than that of African nightshade, spider plant and amaranth.

Table 2.3: Concentrations (g kg^{-1} leaf dry mass) of macro elements taken up as cations in vegetable species (means ± standard deviation). Columns with same lower case letters behind the means indicate no significant differences between means ($p > 0.05$, ANOVA, LSD test).

Species	K	Mg	Ca
	Element concentrations (g kg^{-1} leaf dry mass)		
Exotic kale	36 ± 6 c	4.8 ± 1.3 c	31 ± 6 a
Ethiopian kale	42 ± 8 b	5.1 ± 0.9 c	26 ± 5 b
African nightshade	46 ± 11 ab	6.6 ± 2.3 b	18 ± 6 c
Amaranth	48 ± 8 a	12 ± 2.9 a	32 ± 8 a
Cowpea	32 ± 6 c	5.1 ± 1.3 c	24 ± 5 b
Spider plant	35 ± 11 c	7.4 ± 1.9 b	30 ± 6 a

Calcium concentrations in edible organs varied between 9.0 and 52 g kg^{-1} dry mass (Fig. 2.1c). The range of Ca concentrations, which covered 50% of all data, was generally somewhat smaller than the same range of K concentrations but somewhat larger than the range of Mg concentration (compare the extension of the boxes in Fig. a, b and c). This range of Ca concentrations was largest for exotic kale, amaranth and spider plant, and smallest for Ethiopian kale. The means were 31 g Ca kg^{-1} leaf dry mass for exotic kale, 26 g Ca kg^{-1} leaf dry mass for Ethiopian kale, 18 g Ca kg^{-1} leaf dry mass for African nightshade, 32 g Ca kg^{-1} leaf dry mass for amaranth, 24 g Ca kg^{-1} leaf dry mass for cowpea and 30 g Ca kg^{-1} leaf dry mass for spider plant.The mean Ca concentrations significantly differed among the species from 32 g kg^{-1} leaf dry mass for amaranth to 18 g kg^{-1} leaf dry mass for African nightshade (Table 2.3). The mean Ca concentration of exotic kale was not significantly different from that of amaranth and spider plant, but was significantly higher than that of Ethiopian kale, cowpea and African nightshade.

The Mg and Ca concentrations in our study were similar to those earlier reported by Uusiku et al. (2010), in a review article on unknown amaranth species, African nightshade, cowpea and spider plant. Jiménez-Aguilar and Grusak (2015) found mean concentration of K, Mg and Ca in African nightshade similar to our findings. Similarities with this study were seen in Mg concentrations in amaranth and cowpea and Ca concentrations in cowpea and spider plant (Schönfeldt and Pretorius 2011). Previous study by Kamga et al. (2013), revealed similarities in Mg and Ca concentration in amaranth and African nightshade. Schönfeldt and Pretorius (2011), findings revealed higher Mg concentration in spider plant (1.5 g kg^{-1} fresh mass or 15 g kg^{-1} dry mass) and lower Ca in amaranth (23 g kg^{-1} dry mass). On the contrary, Kruger et al. (1998) found brassica species (kale) to contain 1.3 mg Ca kg^{-1} dry mass while mean Ca concentration in cowpea were higher than previously reported values (Gonçalves et al. 2016). Habwe et al. (2008) showed the vegetables to have low concentration of K and also indicated that spider plant had higher concentration of Ca than amaranth while African nightshade had the highest Mg concentration.

In our study, amaranth had the highest concentrations of K, Mg and Ca among all species (Table 2.3). From a plant nutritional point of view, this raises the question how the positive charge associated with mineral cation accumulation is balanced with negative charge. Nitrogen, P and S do presumably not much contribute to charge balance, because the main part of these elements, which are absorbed by plants in the form of anions, is assimilated to organic compounds without charge (Marschner 2012). Thus, in amaranth, the large positive charge associated with accumulation of cations (3.8 val kg^{-1} dry mass in amaranth in comparison to 2.4 val kg^{-1} dry mass in cowpea) is presumably balanced by high cation exchange capacity of the cell walls ("non-diffusible anions" in the form of negatively loaded carboxylic groups) and by high cellular content of organic acid anions, e.g. citrate and malate.

2.1.4.1.2 Differences among species in the concentration of macro elements absorbed by plants as anions

Concentration of P in edible vegetable organs ranged from a minimum of 2.8 to a maximum 12 g kg^{-1} dry mass (Fig. 2.2a). The range of P concentrations, which covered 50% of all data, was largest in spider plants, while the rest of the vegetables had a smaller range. The mean P concentrations significantly differed among species ranging from 5.2 g kg^{-1} leaf dry mass in cowpea to 12 g kg^{-1} leaf dry mass in spider plant (Table 2.4). The means were 5.3 g P kg^{-1} leaf dry mass for exotic kale, 7.3 g P kg^{-1} leaf dry mass for Ethiopian kale, 7.5 g P kg^{-1} leaf dry mass for African nightshade, 6.2 g P kg^{-1} leaf dry mass for amaranth, 5.2 g P kg^{-1} leaf dry mass for cowpea and 12 g P kg^{-1} leaf dry mass for spider plant.The P concentration of exotic kale did not differ from that of cowpea and amaranth but was significantly lower than that of Ethiopian kale, African nightshade and spider plant. Among the AIVs, P concentration of African nightshade (7.5 g kg^{-1} leaf dry mass) was significantly lower than of spider plant but significantly higher than the P concentration of amaranth and cowpea. A previous study by Jiménez-Aguilar and Grusak (2015) found the mean concentration of P in African nightshade to match with the ranges in our study. The concentration of P in amaranth was similar to that found in South Africa by Odhav et al. (2007). The mean concentration of P in cowpea were higher than in the accessions examined in Ghana by Ahenkora et al. (1998) and that by Chweya et al. 1999, but lower than those found by Imungi et al. (1983), in Kenya. Our study on P concentration of cowpea was in line with the previous study by (Schönfeldt and Pretorius 2011), but disagreed with (Chweya et al. 1999; Abugre et al. 2011; Van Jaarsveld et al. 2014). Also, our study was in line with Abugre et al. (2011) on the concentrations of P in spider plant.

The concentrations of S in the edible plant organs of the vegetables ranged from 1.6 to 19 g/kg dry mass (Fig 2.2b). The range of S concentrations, which covered 50% of all data, was largest for exotic kale, and smallest for amaranth. The means were 11 g S kg^{-1} leaf dry mass for exotic kale, 8.2 g S kg^{-1} leaf dry mass for Ethiopian kale, 4.9 g S kg^{-1} leaf dry mass for African nightshade, 3.9 g S kg^{-1} leaf dry mass for amaranth, 2.4 g S kg^{-1} leaf dry mass for cowpea and 6.4 g S kg^{-1} leaf dry mass for spider plant. The mean concentrations of S varied among species by more than 4 times, from 2.4 g kg^{-1} leaf dry mass for cowpea to 11 g kg^{-1} leaf dry mass in exotic kale (Table 2.4). Among all the vegetable species and in comparison to AIVs, exotic kale had the highest concentration of S.

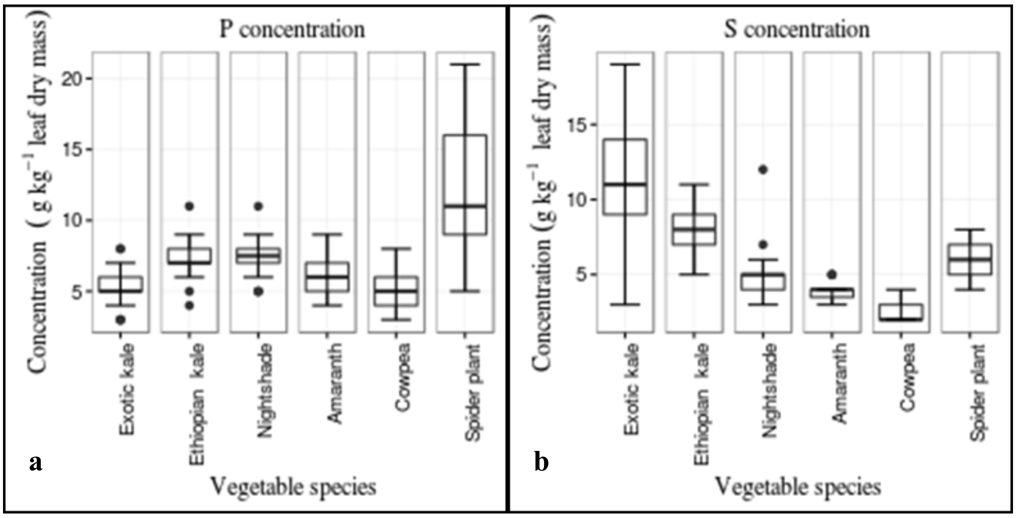

Figure 2.2: Box plots showing minimum, first quartile, median, third quartile and maximum phosphorus (a) and sulphur (b) concentration levels (g kg-1 leaf dry mass) of samples from respective vegetable species. The dots represents (outliers) samples with extreme ranges from the rest, determined by Q3 + 3*IQR or Q1 - 3*IQR formula; where, Q1 – first quartile, Q3 – third quartile, IQR – interquartile range.

Table 2.4 Concentrations (g kg^{-1} leaf dry mass) of macro elements taken up as anions in vegetable species (means ± standard deviation). Columns with same lower case letters behind the means indicate no significant differences between means ($p > 0.05$, ANOVA, LSD test).

Species	P	S
	Element concentrations (g kg^{-1} leaf dry mass)	
Exotic kale	5.3 ± 1.4 d	11.0 ± 3.9 a
Ethiopian kale	7.3 ± 1.5 bc	8.2 ± 1.8 b
African nightshade	7.5 ± 1.3 c b	4.9 ± 1.6 d
Amaranth	6.2 ± 1.3 cd	3.9 ± 0.5 d
Cowpea	5.2 ± 1.2 d	2.4 ± 0.6 e
Spider plant	12 ± 4.7 a	6.4 ± 1.3 c

Comparing the AIVs, Ethiopian kale had the highest S concentrations, followed by spider plant, which was significantly higher than African nightshade and amaranth. The concentration of S in African nightshade and amaranth were higher than in cowpea.

Not so many data are available on the S concentration in vegetable species. Previous study by Jiménez-Aguilar and Grusak (2015), found the mean concentration of S in African nightshade to be higher than in our study. Sulfur is a component of glucosinolates, which are secondary metabolites of *Brassicaceae* including exotic kale, Ethiopian kale and spider plant (Mithen et al. 2010). Thus, the high S concentration in these species is presumably due to their ability to accumulate glucosinolates.

2.1.4.1.3 Concentration of micro elements in vegetable species

Iron concentrations in edible organs varied from a minimum of 92 to maximum of 3531 mg kg^{-1} dry mass (Fig. 2.3a). The range of Fe concentrations, which covered 50% of all data, was generally somewhat smaller than the range of other micro element concentrations (compare the extension of the boxes in Fig. b, c and d). This range of Fe concentrations was largest for Ethiopian kale and amaranth, and smallest for exotic kale. The means were 233 mg Fe kg^{-1} leaf dry mass for exotic kale, 636 mg Fe kg^{-1} leaf dry mass for Ethiopian kale, 711 mg Fe kg^{-1} leaf dry mass for African nightshade, 839 mg Fe kg^{-1} leaf dry mass for amaranth, 640 mg Fe kg^{-1} leaf dry mass for cowpea and 559 mg Fe kg^{-1} leaf dry mass for spider plant. The mean Fe concentrations significantly differed among the species from 839 mg kg^{-1} leaf dry mass for amaranth to 233 mg kg^{-1} leaf dry mass for exotic kale (Table 2.5). Among the AIVs, spider plant (559 mg kg^{-1} leaf dry mass) had a significantly lower Fe concentration than amaranth.

Ethiopian kale, African nightshade and cowpea had lower Fe concentration than amaranth and higher than spider plant, but did not show significant differences.

Concentrations of Zn in the edible plant organs of the vegetables varied from a minimum of 26 to a maximum of 253 mg kg^{-1} leaf dry mass (Fig. 2.3b). The range of concentrations, which covered 50% of all data, was largest for amaranth and smallest for cowpea. The means were 55 mg Zn kg^{-1} leaf dry mass for exotic kale, 83 mg Zn kg^{-1} leaf dry mass for Ethiopian kale, 70 mg Zn kg^{-1} leaf dry mass for African nightshade, 96 mg Zn kg^{-1} leaf dry mass for amaranth, 53 mg Zn kg^{-1} leaf dry mass for cowpea and 80 mg kg^{-1} leaf dry mass for spider plant.

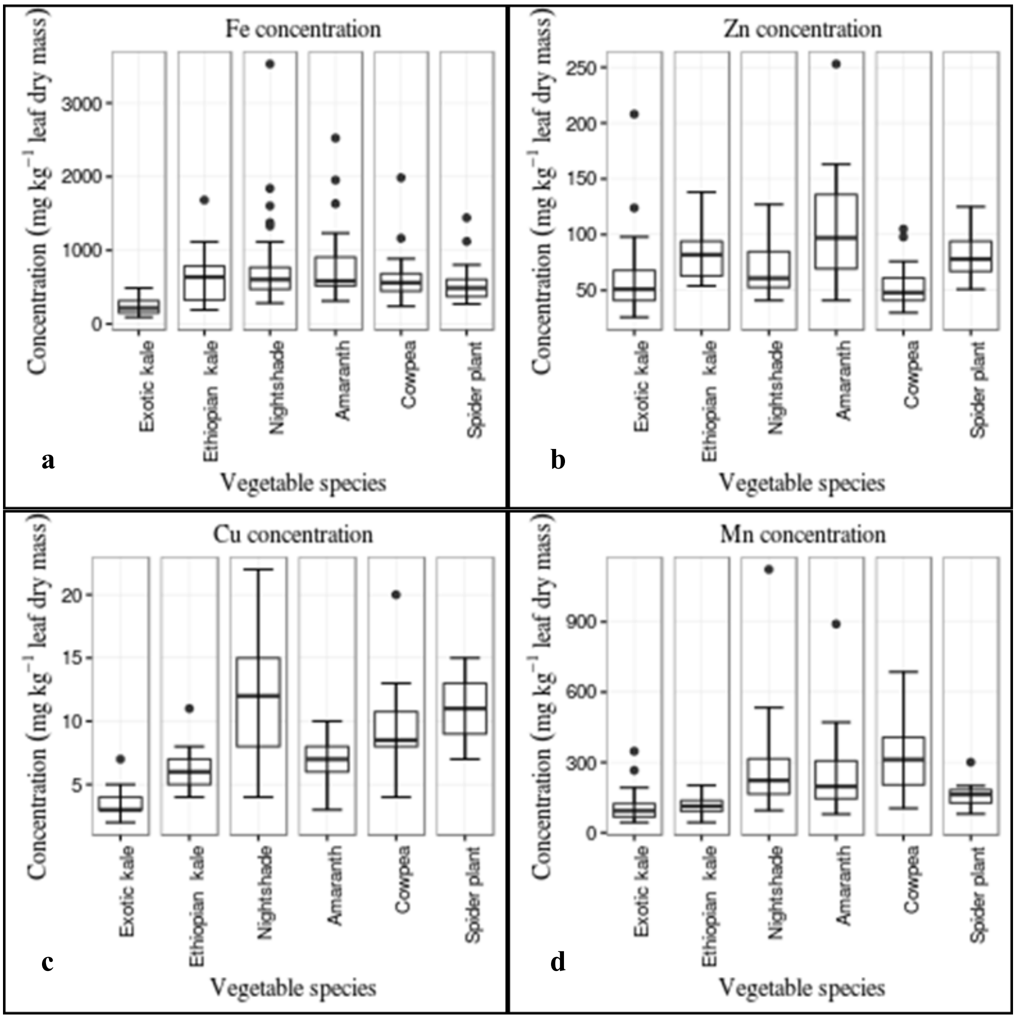

Figure 2.3: Box plots showing, minimum, first quartile, median, third quartile and maximum iron (a), zinc (b), copper (c) and manganese (d) concentration levels (mg kg-1 leaf dry mass) of samples from respective vegetable species. The dots represents (outliers) samples with extreme ranges from the rest, determined by Q3 + 3*IQR or Q1 - 3*IQR formula; where, Q1 – first quartile, Q3 – third quartile, IQR – interquartile range.

The mean Zn concentration significantly differed among species from 96 mg Zn kg^{-1} leaf dry mass for amaranth to 53 mg Zn kg^{-1} leaf dry mass in cowpea (Table 2.3). Exotic kale had the lowest Zn concentration, which was not significantly different from cowpea. No significant differences in Zn concentration were observed between Ethiopian kale and spider plant. On the other hand, African nightshade had a significantly lower concentration of Zn than amaranth.

Copper concentration in edible organs of the vegetables ranged from a minimum of 1.9 to a maximum of 22 mg kg^{-1} leaf dry mass (Fig 2.3c). The range of Cu concentrations, which covered 50% of all data, was largest for African nightshade and smallest for exotic kale.

Table 2.5: Concentrations (mg kg^{-1} leaf dry mass) of micro elements Fe, Zn, Cu and Mn in vegetable species (means ± standard deviation). Columns with same lower case letters behind the means indicate no significant differences between means ($p > 0.05$, ANOVA, LSD test).

Species	Fe	Zn	Cu	Mn
	Element concentrations (mg kg^{-1} leaf dry mass)			
Exotic kale	233 ± 99 c	55 ± 24 c	3.4 ± 1.2 d	109 ± 67 c
Ethiopian kale	636 ± 364 ab	83 ± 24 ab	6.1 ± 1.7 c	116 ± 42 c
African nightshade	711 ± 401 ab	70 ± 23 b	12 ± 4.8 a	238 ± 101 b
Amaranth	839 ± 548 a	96 ± 36 a	6.6 ± 1.9 c	222 ± 119 b
Cowpea	640 ± 363 ab	53 ± 19 c	8.8 ± 2.5 b	332 ± 165 a
Spider plant	559 ± 311 b	80 ± 21 ab	11 ± 2.3 a	159 ± 50 bc

The means were 3.4 mg Cu kg^{-1} leaf dry mass for exotic kale, 6,1 mg Cu kg^{-1} leaf dry mass for Ethiopian kale, 12 mg Cu kg^{-1} leaf dry mass for African nightshade, 6.6 mg Cu kg^{-1} leaf dry mass for amaranth, 8.8 mg Cu kg^{-1} leaf dry mass for cowpea and 11 mg Cu kg^{-1} leaf dry mass for spider plant. The mean Cu concentrations significantly differed among species from 12 mg kg^{-1} leaf dry mass for African nightshade to 3.4 mg kg^{-1} leaf dry mass for exotic kale (Table 2.5). The concentration of Cu in exotic kale was significantly lower than in all AIV species. Among the AIVs, Ethiopian kale (6.1 mg kg^{-1} leaf dry mass) and amaranth had the lowest Cu concentrations. Concentration of Cu in cowpea was significantly lower than in African nightshade and spider plant. African nightshade and spider plant had almost the same concentrations of Cu.

The concentration of Mn in edible organs varied from a minimum of 44 to a maximum of 1124 mg kg^{-1} leaf dry mass (Fig. 2.3d). The range of Mn concentrations, which covered 50%

of all data, was largest for cowpea and smallest for exotic kale. The means were of 109 mg Mn kg^{-1} leaf dry mass for exotic kale, 116 mg Mn kg^{-1} leaf dry mass for Ethiopian kale, 238 mg Mn kg^{-1} leaf dry mass for African nightshade, 222 mg Mn kg^{-1} leaf dry mass for amaranth, 332 mg Mn kg^{-1} leaf dry mass for cowpea and 159 mg Mn kg^{-1} leaf dry mass for spider plant. The mean Mn concentrations significantly differed among species ranging from 109 mg kg^{-1} leaf dry mass for exotic kale to 332 mg kg^{-1} leaf dry mass for cowpea (Table 2.5). The mean Mn concentration of exotic kale was similar to that of Ethiopian kale and spider plant. The Mn concentration of African nightshade was significantly lower than that of cowpea. The Mn concentrations of amaranth, African nightshade and spider plant did not significantly differ.

While the Zn concentration ranges in amaranth and nightshade in this study were within the findings of Kamga et al. (2013), the Fe concentration was lower; 201 and 147 mg kg^{-1} dry mass as compared to 839 and 711, respectively. The concentrations of Zn and Fe in cowpea were similar to Gonçalves et al. (2016), but much higher than those indicated in the study of Frossard et al. (2000). In a similar study done in Dar es Salaam, Tanzania, the range of Zn and Cu concentrations was found to be similar in cowpea and amaranth (Othman 2001). Mutune et al. (2013), did almost a similar study in Kenya and found much higher concentration of copper in spider plant (19 mg kg^{-1} dry mass). The Fe, Zn, Cu and Mn concentrations in African nightshade were higher than those reported in a study on two African nightshade genotypes (*Solanum scabrum,* PI 643126 and Grif 14198) in Central America by Jiménez-Aguilar and Grusak (2015). The overall mean concentration of elements among vegetables was higher in our study than in the study of Fang et al. (2014), who found leafy vegetables collected in China open air and supermarkets to contain average concentrations of 108 mg Fe kg^{-1} dry mass, 29 mg Mn kg^{-1} dry mass, and 36 mg Zn kg^{-1} dry mass.

From the point of view of dietary value, exotic kale for all micro elements was a comparably poor source for intake (Table 2.5). Mean concentration of each micro element differed among AIVs. For example, Ethiopian kale was among the species with the highest concentrations of Zn, but was low in Cu and Mn. Amaranth was among the species with the highest concentrations of Fe and Zn, but not of Cu. In principal, plant micro element concentrations depend on element availability in soil and the ability of plants for element acquisition (Frossard et al. 2000). The differences in micro element concentration among species may also depend on the market types, crop growth seasons and plant traits.

The differences in element concentration was attributed to the fact that plants take up these elements from soil solution in the form of free ions (Fe^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+}), which depend on the soil physiochemical and biological properties (Frossard et al. 2000). The key soil properties related to micro elements availability in the soil are pH, CEC, soil structure and water content. Plants also differ in the root architecture, e.g. distribution of root hairs, root molecular and physiological mechanisms, which determine the volume from which micro elements can be extracted (Marschner 1993), and thus regulate the elements uptake. Changes induced by roots in rhizosphere by plants, regulates uptake of micro elements i.e. Zn. Unlike macro elements (Ca, Mg, K), which are bound to negatively charged cell wall, Fe and Zn are not. This may also apply to Cu and Mn. Iron loading in the plant is facilitated by Fe-chelate, which may differ among plant species (Grusak 1994), while uptake of Cu dependent on plant type rather than soil Cu (Nabulo et al. 2011).

The concentrations of Fe in the vegetables were far higher than those reported in previous studies (Jiménez-Aguilar and Grusak 2015). In the study of Joy et al. (2015), vanadium (V) was used as an indicator for contamination of plant samples with soil particles. In this study, the mean contribution of soil particles to the Fe concentration of (carefully washed) leaves of vegetables was 77%. For specific species, the contribution of soil particles was 97% (Joy et al. 2015). The mean contribution of soil particles to the leaf concentrations of Zn (6%) and Cu (6%) was much lower. The higher levels could be associated to Fe that gets attached to edibles parts through contamination by dust and gets into plant tissue. The Fe concentration is thus not entirely bioavailable to human beings.

The Al concentrations in the vegetables were determined in order to reveal the source of excess Fe concentration in vegetables. Al translocation from roots to shoots in most plants is usually insignificant (Klug et al. 2015). Thus the measured Al in vegetables/ crops originates from the soil/ 'dust'. A significant positive correlation between Al and Fe was a clear indication that excess Fe in the vegetable species was a result of atmospheric contamination (Fig. 2.4). The coefficient of determination indicated that 95% of the samples showed a positive relationship between Al and Fe as opposed to other elements. The coefficient of determination (r^2) for other elements were below 0.4 as follows; 0.11 for Zn, 0.24 for Cu, 0.34 for Mn, 0.39 for K, 0.018 for Ca, 0.27 for Mg, 0.17 for P and 0.29 for S. Since a fraction of Fe concentration in vegetables species was a result of atmospheric deposition, washing of the samples was done to determine the amount of Fe that can be eliminated and the species that accumulated a lot of aerial deposited Fe concentration.

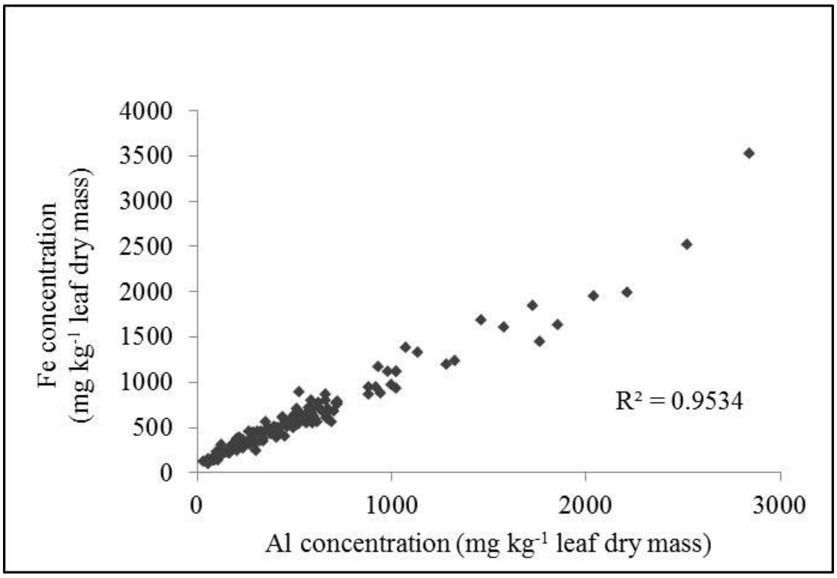

Figure 2.4: Relationship between Al and Fe concentrations (mg kg^{-1} leaf dry mass) vegetable samples, n = 140; R^2 = coefficient of determination.

Comparison was done between the unwashed and washed samples in order to find out the differences in the Fe concentration among vegetables species. This study found out that, washing was able to remove a range of 24% - 60% of Fe adhered to the edible vegetable parts (Table 2.6). Fe concentration in exotic kale was least removed. The order of removal of the Fe concentrations was exotic kale < Ethiopian kale/amaranth < African nightshade < cowpea < spider plant. Zn and Cu concentration were not reduced by washing of vegetables species whereas little reduction of Mn concentration was observed in all the vegetable species apart from exotic kale.

Table 2.6: Reduction of micro element concentrations in percent (%) by washing vegetable species with water (means ± standard error of means).

Species	Fe	Zn	Cu	Mn
	Percentage (%) element concentration			
Exotic kale	24 ± 4	-5.3 ± 4	-6.4 ± 5	-4.6 ± 5
Ethiopian kale	38 ± 6	-18 ± 8	-12 ± 3	12 ± 8
African nightshade	47 ± 4	-1.8 ± 3	-8.2 ± 5	11 ± 4
Amaranth	38 ± 6	0.02 ± 3	0.4 ± 3	12 ± 5
Cowpea	50 ± 3	-0.6 ± 3	-7 ± 4	7.5 ± 4
Spider plant	60 ± 6	5.4 ± 4	0.2 ± 5	28 ± 8

The reduction observed in Fe was in agreement with Sharma et al. (2008b), who discovered reductions at production sites to be less than 10% while reductions at the market sites varied from 23 to 68% in micro and heavy metal concentrations. These results disagreed with Sharma et al. (2007) who found washing vegetables with water to have reduced the levels of Zn and Cu concentrations in the vegetables. The effects of washing indicated that atmospheric deposition contributed immensely to the increased levels of Fe in vegetables (Sharma et al. 2008a), which suggest that urban atmospheric contamination as a result of automobile and accumulated dust may be a major source of heavy metals.

2.1.4.2 Effects of markets on essential element concentrations in vegetable species

To determine the effect of market type on element concentration of vegetable species, unwashed data were used. The data for both seasons within respective markets were combined and analysed to get the mean concentrations. Comparisons between markets were done using unpaired T-tests.

2.1.4.2.1 Effects of markets on macro element concentrations in vegetables species

Vegetable species varied in their concentrations between open air and supermarkets, however there were no significant differences between markets in most element concentrations of vegetables (Table 2.7). There were no differences in K concentrations between markets in all the vegetables species. The concentration of Ca and Mg was higher in exotic kale collected from the supermarkets (33 and 5.4 g kg^{-1} leaf dry mass, respectively) than open air markets (28 and 4.3 g kg^{-1} leaf dry mass, respectively). On the other hand, supermarkets showed the highest concentrations of P in exotic kale and amaranth compared to open air markets, whereas cowpea found in the supermarket had the highest concentrations of S (2.8 g kg^{-1} leaf dry mass) unlike in the open air market where S concentrations were as less as 0.7 g kg^{-1} leaf dry mass. All the macro element concentrations in Ethiopian kale and spider plant were the same in open air markets and supermarkets.

Little study has been done in the past to determine the differences in macro element concentrations in vegetables between markets. Most of the studies done concentrated on the vegetables grown in the field. In case of market study, some research concentrated on pathogen contamination (Newman 2005; Mohamed et al. 2016), micro nutrients and heavy metal concentrations in vegetables.

In our study, exotic kale collected from supermarkets had the highest concentrations of P, Ca and Mg compared to exotic kale collected from open air market. The presence of higher levels of Ca, Mg and P in kale from supermarket was an indication of preference given to exotic kales that are produced for the supermarkets by use of fertilizer as opposed to AIVs.

Table 2.7: Effects of market type on concentrations (g kg^{-1} leaf dry mass) of macro elements in vegetable species (means ± standard deviation); where XK – exotic kale, EK – Ethiopian kale, AN - African nightshade, AM - amaranth, CP - cowpea and SP - spider plant. Rows with same lower case letters behind the means indicate no significant differences between means of a respective element ($p > 0.05$, ANOVA, unpaired t-test, $n = 10$ for open air markets, $n = 5$ for supermarkets).

Species	K		Mg		Ca		P		S	
	Open air market	Supermarket	Open air market	Supermarket	Open air market	Supermarket	Open air market	Supermarket	Open air market	Supermarket
	Element concentrations (g kg^{-1} leaf dry mass)									
XK	32 ± 6 a	33 ± 6 a	4.3 ± 1.1 a	5.4 ± 1.2 b	28 ± 8 b	33 ± 4 a	4.8 ± 1 b	5.8 ± 1 a	10.2 ± 3.0 a	9.8 ± 5.3 a
EK	41 ± 9 a	42 ± 12 a	4.8 ± 0.8 a	4.9 ± 1.3 a	25 ± 5 a	21 ± 5 a	6.6 ± 1 a	7.8 ± 2 a	7.6 ± 2.2 a	8.6 ± 0.7 a
NS	44 ± 10 a	44 ± 11 a	6.7 ± 2.6 a	5.4 ± 1.3 b	16 ± 3 a	19 ± 7 a	7.3 ± 1 a	7.1 ± 2 a	4.2 ± 0.8 a	4.7 ± 2.0 a
AM	43 ± 9 a	49 ± 7 a	12 ± 3.1 a	11 ± 1.5 a	29 ± 4 a	29 ± 6 a	6.0 ± 1 b	7.2 ± 1 a	3.4 ± 0.6 a	3.6 ± 0.7 a
CP	29 ± 6 a	32 ± 4 a	5.0 ± 1.4 a	4.8 ± 0.9 a	22 ± 4 a	25 ± 4 a	4.8 ± 1 a	5.3 ± 1 a	2.1 ± 0.4 b	2.8 ± 0.6 a
SP	34 ± 8 a	47 ± 16 a	6.9 ± 1.6 a	7.9 ± 0.5 a	26 ± 6 a	32 ± 5 a	10.6 ± 3 a	16.0 ± 2 a	6.0 ± 1.3 a	6.6 ± 0.7 a

In the previous study, Cunnigham et al. (2001), suggested that element contents in vegetables are affected by soil type and conditions like fertilizer type and application among other factors. It was expected that vegetables bought in supermarkets will contain high levels of macro elements based on the fact that farmers use good agronomic measures while producing vegetables for supermarket supply. A survey by RSA (2015), showed that most consumers prefer buying vegetables in the supermarket (45%) because they believe the vegetables are of good quality while 36% who buy from open air markets cited closeness to their offices or homes and easy accessibility as reasons to buy there.

2.1.4.2.2 Effects of market on micro element concentration in vegetable species

Although vegetable species varied in micro element concentrations, there were no significant differences observed between markets in most vegetables ($p<0.05$, LSD post hoc test; Table 2.8). Mean Fe concentration was higher in exotic kale, Ethiopian kale, amaranth and spider plant collected from the supermarket but there were no significant differences between the two markets under the above specified vegetables. On the other hand, spider plant, among all the species, showed significant differences in Zn concentrations between markets, where the concentrations were higher in the supermarket (104 mg kg^{-1} leaf dry mass). The concentration of Zn in exotic kale, Ethiopian kale, African nightshade, amaranth and cowpea did not have differences between markets. No significant differences between markets were observed in the concentration of Cu and Mn in all the vegetable species.

The results of our study were in agreement with Fang et al. (2014) who found Fe, Zn, Mn and Cu concentrations in leafy vegetables from traditional markets and supermarkets, in Beijing China, to be relatively equal. The amount of Zn in vegetables found in both markets was higher than in the study by Li et al. (2006) in China. Our study findings suggest that micro elements are not affected by the market type, as it was observed with macro elements. It was expected that open air markets will have higher levels of Fe and to some extent Zn and Cu due to the exposed nature of the market that is prone to dust and contamination (Abdu et al. 2011). This doesn’t rule out the issue of dust contamination in vegetables. Vegetables in the supermarkets and open markets were both contaminated with Fe equally since the concentration levels were much higher than the dietary intake recommended values and what other studies have found out. Contamination by dust was looked into more detail in the previous sections.

Table 2.8: Effect of market type on concentrations (mg kg^{-1} leaf dry mass) of micro elements in vegetable species (means ± standard deviation); where XK – exotic kale, EK – Ethiopian kale, AN - African nightshade, AM - amaranth, CP - cowpea and SP - spider plant. Rows with same lower case letters behind the means indicate no significant differences between means of a respective element ($p > 0.05$, ANOVA, unpaired T-test, n = 10 for open air markets, n = 5 for supermarkets).

Species	Fe		Zn		Cu		Mn	
	Open air market	Supermarket	Open air market	Supermarket	Open air market	Supermarket	Open air market	Supermarket
	Element concentrations (mg kg^{-1} leaf dry mass)							
XK	317 ± 164 a	352 ± 191 a	57 ± 23 a	47 ± 13 a	3.2 ± 1 a	3.4 ± 1 a	101 ± 49 a	101 ± 26 a
EK	1137 ± 600 a	1184 ± 924 a	70 ± 17 a	79 ± 21 a	5.5 ± 1 a	5.5 ± 1 a	128 ± 49 a	137 ± 49a
NS	1771 ± 1201 a	1403 ± 1177 a	68 ± 18 a	67 ± 13 a	12.3 ± 5 a	10.1 ± 4 a	315 ± 197 a	281 ± 158a
AM	1095 ± 569 a	1985 ± 1032 a	94 ± 34 a	106 ± 44 a	7.0 ± 2 a	6.9 ± 1 a	301 ± 232 a	244 ± 89 a
CP	1536 ± 1369 a	1415 ± 730 a	51 ± 23 a	63 ± 15 a	8.8 ± 2 a	9.4 ± 5 a	335 ± 190 a	457 ± 224 a
SP	1497 ± 979 a	4523 ± 1152 a	78 ± 23 b	104 ± 10 a	10.5 ± 3 a	13.6 ± 3 a	222 ± 132 a	478 ± 95 a

2.1.4.3 Effect of seasons on concentration of essential elements in vegetable species

The seasons influence on element concentrations were determined by combining the data from both markets for a particular season belonging to a given vegetable species.

2.1.4.3.1 Effects of seasons on macro element concentrations in vegetable species

Vegetable species differed in the concentration of macro elements between seasons (Table 2.9). The concentration of K in all the vegetable species showed significant differences between seasons. Wet season vegetables had the highest concentration of K in exotic kale at 35 g kg^{-1} leaf dry mass, Ethiopian kale at 47 g kg^{-1} leaf dry mass, African nightshade at 57 g kg^{-1} leaf dry mass, amaranth at 52 g kg^{-1} leaf dry mass, cowpea at 32 g kg^{-1} leaf dry mass and spider plant at 44 g kg^{-1} leaf dry mass. Only cowpea was significantly highest in Mg concentration (5.6 g kg^{-1} leaf dry mass), in the dry season compared to wet season (4.3 g kg^{-1} leaf dry mass). Exotic kale was significantly high in Ca in the dry season (32 g kg^{-1} leaf dry mass) and low in wet season (27 g kg^{-1} leaf dry mass) while other vegetables did not show differences between seasons. All the vegetable species had the same amount of P concentration between the two seasons. On the other hand, amaranth, cowpea, exotic kale and spider plant showed differences between seasons in S concentrations.

The concentrations of S in amaranth and spider plant were higher in wet season (3.7 and 7.0 g kg^{-1} leaf dry mass, respectively) compared to dry season (3.2 and 5.5 g kg^{-1} leaf dry mass, respectively). On the other hand, cowpea and exotic kale showed higher concentrations of S in dry season (2.5 and 12 g kg^{-1} leaf dry mass, respectively) than wet season (2.1 and 8.6 g kg^{-1} leaf dry mass, respectively).

This study showed that seasons affected vegetables species differently with respect to element concentrations. This is partially in agreement with Ziblim et al. (2012), who found an Ethiopian indigenous grass (*Pennisetum pedicellatum*) to be high in K and P in the wet season whereas there were no differences between Mg and Ca in the wet and the dry season. Another study by Bryla and Duniway (1997) found safflower (*Carthamus tinctorius*) and wheat (*Triticum aestivum*) to accumulate higher levels of P in well-watered soils than in dry soils.

Table 2.9: Effect of seasons on concentrations (g kg^{-1} leaf dry mass) of macro elements in vegetables species (means ± standard deviation); where XK – exotic kale, EK – Ethiopian kale, AN - African nightshade, AM - amaranth, CP - cowpea and SP - spider plant. Rows with same lower case letters behind the means indicate no significant differences between means of a respective element ($p > 0.05$, ANOVA, unpaired T-test).

Species	K		Mg		Ca		P		S	
	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season
	Element concentrations (g kg^{-1} leaf dry mass)									
XK	30 ± 5 b	35 ± 6 a	5.0 ± 1.4 a	4.3 ± 0.9 a	30 ± 8 a	30 ± 6 a	5.1 ± 1.4 a	5.2 ± 0.7 a	12 ± 4.0 a	8.6 ± 3.2 b
EK	35 ± 8 b	47 ± 7 a	4.8 ± 0.9 a	4.7 ± 0.9 a	22 ± 5 a	25 ± 5 a	6.6 ± 1.4 a	7.2 ± 1.4 a	7.8 ± 1.7 a	8.0 ± 2.2 a
NS	37 ± 4 b	51 ± 10 a	5.9 ± 0.8 a	6.6 ± 2.9 a	17 ± 6 a	16 ± 4 a	7.1 ± 1.4 a	7.3 ± 1.1 a	4.4 ± 1.7 a	4.3 ± 0.9 a
AM	38 ± 5 b	52 ± 5 a	13 ± 1.5 a	11 ± 3.4 a	32 ± 4 a	27 ± 3 b	6.4 ± 1.0 a	6.4 ± 1.6 a	3.2 ± 0.6 b	3.7 ± 0.5 a
CP	28 ± 4 b	32 ± 6 a	5.6 ± 1.1 a	4.3 ± 1.1 b	22 ± 5 a	24 ± 3 a	5.2 ± 0.7 a	4.7 ± 1.2 a	2.5 ± 0.6 a	2.1 ± 0.4 b
SP	30 ± 3 b	44 ± 11 a	6.9 ± 1.4 a	7.1 ± 1.9 a	25 ± 3 a	29 ± 8 a	12 ± 3.1 a	11 ± 4.6 a	5.5 ± 0.8 a	7.0 ± 1.3 b

Higher concentrations of K during wet season were expected based on the diffusion and soluble nature of the element. Uptake of K by the plants is highly depended on soil moisture. Increase in soil moisture leads to increased movement of K to plants and hence higher availability. Since P movement to plants is also dependent on soil moisture, it was expected that the trend will be the same in this study, unless the P in the soil was adsorbed. Despite moisture being a driving force in P availability for plants, its use by plants mainly depends on supplied P form and ability of the plant to take up the available P. The limitation in the study was that soil samples were not collected for analysis in areas where these vegetables were grown.

2.1.4.3.2 Effects of seasons on micro element concentrations in vegetable species

Differences between seasons were observed only in Fe concentrations (Table 2.10). Exotic kale, Ethiopian kale and African nightshade (1429, 2203 and 392 mg Fe kg^{-1} leaf dry mass, respectively) recorded higher concentrations of iron in the wet season unlike in the dry season. No difference between seasons was observed in iron concentrations in amaranth, cowpea and spider plant. There were no seasonal changes in Zn, Cu and Mn concentrations among vegetable species.

The results of this study agrees with a previous study by Birnin-Yauri et al. (2011), who found the values for the micro elements higher in leafy vegetable samples during the rainy season. Also, Angle et al. (2003) found zinc alongside nickel to be highly accumulated in leaves of hyper accumulators at 80% and 100% water holding capacity. On the contrary, Sharma et al. (2009), found the concentration of Zn and Cu higher in summer than winter, Sharma's arguments are based on high atmospheric contamination during the summer period.

In this study, high concentration of Fe during the wet season may be as a result of aerial deposits during rainfall events or as a result of soil contamination since at most cases wet soils are likely to be attached on plant leaves during harvest. Another reason could be the sprinkling of water to vegetables at the market place to keep them fresh leading to minute aerial deposits sticking on the vegetables (Sharma et al. 2009).

Table 2.10: Effect of seasons on concentrations (mg kg^{-1} leaf dry mass) of micro elements in vegetable species (means ± standard deviation); where XK – exotic kale, EK – Ethiopian kale, AN - African nightshade, AM - amaranth, CP - cowpea and SP - spider plant. Rows with same lower case letters behind the means indicate no significant differences between means of a particular element (p > 0.05, ANOVA, unpaired T-test).

Vegetables	Fe		Zn		Cu		Mg	
	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season
	Element concentrations (mg kg^{-1} leaf dry mass)							
XK	271 ± 180 b	392 ± 143 a	53 ± 21 a	55 ± 20 a	3.1 ± 1 a	3.5 ± 1 a	93 ± 45 a	110 ± 38 a
EK	844 ± 517 b	1429 ± 716 a	70 ± 20 a	75 ± 17 a	5.2 ± 1 a	5.8 ± 2 a	114 ± 41 a	146 ± 50 a
NS	1017 ± 518 b	2203 ± 1332 a	69 ± 17 a	67 ± 16 a	12 ± 3 a	12 ± 5 a	283 ± 212 a	322 ± 159 a
AM	1537 ± 1009 a	1218 ± 634 a	107 ± 40 a	89 ± 33 a	7.7 ± 1 a	6.2 ± 2 a	360 ± 247 a	212 ± 106 a
CP	1367 ± 627 a	1634 ± 1610 a	51 ± 13 a	57 ± 28 a	8.6 ± 3 a	9.3 ± 3 a	339 ± 185 a	402 ± 224 a
SP	2074 ± 1193 a	1640 ± 1824 a	86 ± 22 a	74 ± 25 a	11 ± 3 a	10 ± 3 a	276 ± 156 a	226 ± 159 a

2.1.5 General discussion and recommendation

We found that the mean element concentrations in edible organs across market types and growing seasons were significantly different among species (Tables 2.3, 2.4 and 2.5). In the general discussion, we discuss two questions: 1) to what extent can our data concerning differences among species in the concentrations of macro- and micro elements be generalized? The discussion of this question is relevant for any comparative statements concerning the nutritional value of specific species, e.g. AIV-species in comparison to "exotic" vegetable species. Must these statements be confined to the specific context of our investigation, i.e., markets in Nairobi, or can statements about the nutritional value of specific species relative to that of other species be generalized? 2) Can AIV substantially contribute to dietary element supply? The discussion of this question is relevant for assessing the potential contribution of AIV for prevention of insufficient dietary mineral element intake, and thus for reducing "hidden hunger".

2.1.5.1 To what extent can our data concerning differences among species in the concentrations of macro and micro elements be generalized?

It is well documented, that the plant mineral concentration is dependent on environmental conditions that control soil nutrient availability, e.g., soil nutrient content and soil moisture content, and on other environmental conditions that control leaf growth, and thus growth-related "dilution" of leaf nutrients (Marschner 2012; Dickinson et al. 2014). Accordingly, previous studies have shown that mineral nutrient concentrations in leafy vegetables vary, depending on soil nutrient content (Kiba et al. 2012; Joy et al. 2015). The samples included in our study were collected from 15 markets in each of two seasons. It is not known under which specific site conditions the vegetables included in our study were cultivated. In Kenya, no species-specific fertilizer recommendations exist, and there are no recommendations for growing specific species under specific site conditions. Thus, there is no reason to assume that specific species were mainly cultivated under specific soil fertility conditions. For that case, it was assumed that, the soil fertility conditions did not differ among species. Thus, the differences in the mean mineral concentrations among species (Tables 2.3, 2.4, 2.5) were primarily due to plant traits, which control leaf mineral concentrations, and not due to different site conditions.

With regard to the concentrations of macro elements, which are absorbed by plants in the form of cations, the most prominent result was the very high Mg concentration in amaranth (Table 2.3). This is in line with other studies in which several AIVs were grown under identical site conditions (Kamga et al. 2013; see also chapter 4 of this thesis). In amaranth, the concentrations of other cationic macro nutrients were also high (Table 2.3), whereas concentrations of macro nutrients, which are absorbed and at least in part accumulated in the form of anions, were not particularly high (Table 2.4). This indirectly points to the cation exchange capacity of cell walls and/or organic acid anion metabolism and accumulation as possible causes for the high concentrations of cationic macro elements in amaranth.

With regard to the concentrations of macro elements, which are absorbed by plants in the form of anions, the most prominent results are the very high S concentrations in the exotic and Ethiopian kales, followed by spider plant, and the very high P concentrations in spider plants (Table 2.4). The kales and spider plant belong to the *Brassicaceae*, a family which is containing many species, which accumulate sulphurous glucosinolates (Mithen et al. 2010). In a recent study glucosinolate accumulation was confirmed in all tested spider plant genotypes (Omondi et al. 2017). Higher P concentrations in spider plant in comparison to other AIV and exotic kale are in line with data from Jiménez-Aguilar and Grusak (2015), and our own data reported in chapter 3 and 4 of this thesis, which were derived from experiments, in which all species were cultivated under identical site conditions. Our data on internal P utilization efficiency of spider plants in comparison to other species (chapter 3) point to high metabolic P demand of spider plant as a possible cause for the high P concentrations in edible organs. Thus, we assume that our data showing that in comparison to other AIV the concentrations of S in the kales and spider plant, and P in spider plant are particularly high can be generalized.

With regard to micro elements, Zn and Fe have an outstanding role for the nutritional value (Frossard et al. 2000; Lim et al. 2012; Kumssa et al. 2015b). In our study, Zn concentrations were particularly high for amaranth, Ethiopian kale and spider plant, and particularly low for cowpea and exotic kale (Table 2.5). In line with these results, higher Zn concentrations in spider plant in comparison to nightshade were also found by Jiménez Aguilar and Grusak 2015 (in comparison to nightshade). In the study of van Jaarsveld et al. (2014), spider plant had the highest leaf Zn concentrations among 8 leafy African vegetable species. Lower Zn concentrations in cowpea than in amaranth and spider plant were also found by Schönfeldt and Pretorius (2001), Uusiku et al. (2010) and Kruger et al. (2015). Thus, there is some support from other studies, in which the species were cultured under identical site conditions that Ethiopian kale, spider plant and amaranth have particularly high Zn concentrations in edible organs.

Fe concentrations showed very large variation within each species, and were significantly higher in the AIVs than in exotic kale (Table 2.5). Higher Fe concentrations in leaves of AIV species in comparison to exotic kale is in line with our data from two field experiments (Nambafu et al. non-published results), in which all six species were cultivated under identical site conditions. In the study of van Jaarsveld et al. (2014), leaf Fe concentrations of AIV species were also higher than that of Chinese cabbage (*Brassica rapa* L. subspecies *chinensis*). Iron concentrations in leaves are strongly influenced by external contamination with soil particles (Table 2.6; Joy et al. 2015). Thus, higher leaf Fe concentrations in AIV in comparison to exotic kale may possibly result from morphological and anatomical leaf traits, e.g. fleshy leaves with low surface to volume ratio, that lead to lower surface contamination with Fe containing particles.

2.1.5.2: Can AIVs substantially contribute to dietary element supply?

Concerning the dietary element supply we confine our discussion to the macro elements Mg and Ca, and the micro elements Fe and Zn, as low supply of these elements is generally considered to play a prominent role for malnutrition in sub-Sahara Africa (Lim et al. 2012; Joy et al. 2014; Kumssa et al. 2015a, 2015b). For these elements, age- and sex-specific values for recommended daily intake (RDI) were given in a joint report of FAO and WHO (2001; Table 2.11). The daily recommended nutrient intakes for adults are 13.7 mg Fe, 14.0 mg Zn, 260 mg and 1000 mg Ca for males, and 29.4 mg Fe, 9.8 mg Zn, 220 mg and 1000 mg Ca for females.

Table 2.11: Recommended daily nutrient intakes (RNI) of elements for different age groups and sexes (FAO/WHO 2001); data for Zn are based on food with low Zn bioavailability; data for Fe are based on food with 10% Fe bioavailability.

Age (years)	Sex	Fe (mg)	Zn (mg)	Mg (mg)	Ca (mg)
1 - 3		5.8	8.3	60	500
4 - 6		6.3	9.6	76	600
7 - 9		8.9	11.2	100	700
10-18	Male	17	17.1	230	1300
	Female	31.9	14.4	220	1300
19 - 65	Male	13.7	14	260	1000
	Female	29.4	9.8	220	1000

Source: Uusiku et al. (2010)

In the study of Ruel et al. (2005), it is stated that the average vegetable intake in Kenya is 2.88 kg fresh mass/person/week for the rural population and 1.46 kg fresh mass/person/week for the urban population. We assumed a daily vegetable dish of 400 g fresh mass. For assessing the potential contribution of leafy vegetables to dietary element supply, we used the mean concentrations of Mg, Ca (Table 2.3), Fe and Zn (Table 2.5). As these concentrations are given on the basis of dry mass, we used our data on species-specific dry matter percentage in edible organs from our field experiment (see Chapter 4) to calculate the dietary supply of Mg, Ca, Fe and Zn, which is associated with consumption of 400 g fresh mass of various vegetable species (Table 2. 12). Finally, from the data in Table 2.12, and the data on RDI in Table 2.11, we calculated the percentage of RDI, which is supplied through daily consumption of 400 g fresh mass of the different species (Table 2.13).

Table 2.12: Potential contribution of vegetable species to dietary element supply (mg) per daily dish of 400 g fresh mass of various vegetables; calculation of dietary element supply is based on the mean values across markets and growing seasons (Tables 2.3 and 2.5) and the assumption of a dry matter content of edible organs of 11% for exotic and Ethiopian kale, 8% for African nightshade, 13% for amaranth, and 14% for cowpea and spider plant.

Species	Fe	Zn	Mg	Ca
	Dietary element supply (mg per 400 g fresh mass)			
Exotic kale	10	2.4	211	1364
Ethiopian kale	28	3.7	224	1144
African nightshade	23	2.2	211	576
Amaranth	44	5.0	624	1664
Cowpea	36	3.0	286	1344
Spider plant	31	4.5	414	1680

Leafy vegetables may substantially contribute to the RNI, whereby large differences existed depending on which nutrient is considered and which species is consumed (Tables 2.12 and 2.13). With regard to micro element supply, our data indicate that leafy vegetables may contribute more to dietary supply of Fe than Zn. For example, in average, a daily 400 g dish of all the vegetables species would cover 97% (ranging from 34% with consumption of exotic kale to 150% with consumption of amaranth) of the recommended intake of Fe for females, but only 35% (ranging from 22% with African nightshade to 51% with amaranth) of the recommended intake of Zn. Lower dietary supply of Zn than of Fe associated with consumption of AIV has also been reported in other studies (van Jaarsveld et al. 2014; Kruger et al. 2015; van der Hoeven et al. 2016) indicating that leafy vegetables are a poorer source of Zn than of Fe.

Table 2.13: Potential contribution (percentage) of a daily dish of 400 g fresh mass of various vegetable species to the daily recommended intake of mineral elements (RNI); RNI values of male and female adults between the age of 19 and 65 years, and the data from table 2.12 were used for calculation of the contribution.

Species	Fe		Zn		Mg		Ca	
	Male	Female	Male	Female	Male	Female	Male	Female
	Contribution of daily vegetable dish to RNI (% of RNI)							
Exotic kale	73	34	17	24	81	96	136	136
Ethiopian kale	204	95	26	38	86	102	114	114
African nights.	168	78	16	22	81	96	58	58
Amaranth	321	150	36	51	240	284	166	166
Cowpea	263	122	21	31	110	130	134	134
Spider plant	226	105	32	46	159	188	168	168
ø all species	**209**	**97**	**25**	**35**	**126**	**149**	**129**	**129**

For dietary supply of elements, not only the amount of element intake but also the bioavailability (termed also "bioaccessibility") of elements in the food is important. The values for the contribution of a daily vegetable dish of 400 g fresh mass to the recommended Fe intake (Table 2.13) are based on a bioavailability of 10%. In a study on 13 crude leafy vegetables in India, the bioavailability of Fe as assessed by an *in vitro* test, varied between 6 and 44% (Fairweather-Tait et al. 2007).

Bioavailability of Fe depends on both, host factors, e.g., the iron status or infection with malaria (Cercamondi et al. 2010), and food factors, i.e. the content of inhibitors and promotors of Fe absorption (Ancueanau et al. 2015; Icard-Verniere et al. 2016). The content of inhibitors, e.g. phytate, and promotors, e.g. ascorbic acid, in turn, is dependent not only on species-specific plant traits, but also on post-harvest technology and food processing (Uusiku et al. 2010; Grefeuille et al 2011; Gupta et al. 2013). It is often assumed, that Fe bioavailability in vegetables in comparison to cereals is high due to high contents of ascorbic acid and low phytate contents (Frossard et al. 2000). However, in leafy vegetables, external Fe from dust and soil particles may substantially contribute to the Fe content. In our study, the close relationship between Fe- and Al-concentrations clearly indicated external contamination of carefully washed samples with Fe from soil (Fig. 2.4). In the study of Joy et al. (2015), the mean contribution of soil particles to the Fe concentration of (carefully washed) leaves of vegetables was 77%. For specific species, the contribution of soil particles was up to 97% (Joy et al. 2015).

There is conflicting evidence for bioavailability of soil Fe for humans. In a study about geophagy (soil eating) of pregnant women in Tanzania, it was found that soil consumption was associated with an increased risk of Fe deficiency-induced anaemia, suggesting that soil Fe is not bioavailable (Kawai et al. 2009). This is in agreement with other studies that show that soil ingestion can potentially reduce the absorption of already bioavailable micro elements (Hooda et al. 2004). In a recent study, however, it was found that external Fe from contamination with acidic soil but not calcareous soil can make small contribution to Fe nutrition (Gibson et al. 2015). Irrespectively, whether external Fe from soil contamination has negative effects on bioavailability of internal plant Fe or can make small positive contribution to Fe nutrition, we assume that our data in Table 2.13, which are based on a Fe bioavailability of 10% overestimate the true potential contribution of vegetable consumption to Fe supply for humans strongly. Thus, for assessment of potential contribution of leafy vegetables to dietary Fe supply, more knowledge on bioavailabilty of Fe is needed.

For the other elements (Zn, Mg, Ca), the contribution of external soil contamination to the measured element concentrations is expected to be substantially lower, as the concentrations of these elements in soil are much lower. In line with this expectation, Joy et al. (2015) found that the contribution of soil contamination with these elements was less than 6%. Therefore, we assume that our data in Table 2.13 do not strongly overestimate the true potential contribution of vegetable consumption to the dietary supply of Zn, Mg and Ca.

2.2 Nutritional value of AIVs: Heavy metal concentrations of samples from open air and supermarkets in Nairobi

2.2.1 Abstract

Problems in food safety exist as a result of heavy metals accumulation in leafy vegetables in urban and peri-urban small holder systems in sub Saharan Africa (sSA). Heavy metals like cadmium (Cd) and lead (Pb) accumulation in plants discredits the nutritional and health aspects found in leafy vegetables. Vegetables may be contaminated by heavy metals through superficially adhering dust on edible surfaces or root uptake from soil and internal distribution to edible organs. This study was done to measure heavy metals in edible plant organs of vegetables sold on markets in Nairobi in order to assess potential health risks associated with consumption of AIVs. Fresh samples of AIVs (African nightshade - *Solanum scabrum*, amaranth - *Amaranthus cruentus*, cowpea - *Vigna unguiculata*, spider plant - *Cleome gynandra*, Ethiopian kale - *Brassica carinata*) and a standard species commonly grown in Kenya (exotic kale - *Brassica oleracea acephala* group) were collected from ten open air and five supermarkets in Nairobi. Before elements were analysed, the edible parts of the vegetables were washed with normal water and rinsed with distilled water to remove elements associated with externally adhering soil /dust or not washed at all. Two heavy metals, namely Cd and Pb, where measured. The results showed that heavy metal concentrations were similar in samples from open air and supermarkets. Cd concentrations in almost all the vegetables, apart from spider plant and Ethiopian kale, were higher in the dry season compared to the wet season. About 98% of the samples did not exceed 2 mg Cd kg^{-1} dry mass, indicating that vegetables may be safe for consumption. Washing with water strongly reduced Pb concentrations (up to 59% for spider plant), indicating that external adhering dust or soil can be a major source of heavy metals in vegetables.

Keywords: Heavy metals, health risks, leafy vegetables, surface contamination

2.2.2 Introduction

2.2.2.1 Heavy metals, their sources and effects to humans

Urban population is projected to increase to nearly a half of the total population by 2030 (UN 2014), this means that most of the food crops, vegetables included, produced will be grown purposely to feed urban dwellers. However, heavy metal contamination in vegetables and other food stuffs may be a threat to food safety especially in urban and peri-urban areas in sub-Saharan Africa. Heavy metals, like some heavy metals, are naturally occurring metallic elements with densities greater than $5 g/cm^3$, which are toxic to humans, e.g. Cd and Pb (Tchounwou et al. 2012). Elements like Cd and Pb are of environmental concern due to their cumulative behavior in plants and human tissues (Stylianou et al. 2007; Taiwo et al. 2014) since they are not degradeable (Lasat 1997; Gall and Rajakaruna 2013).

Chronic or acute accumulation of heavy metals in the human body may result in cancer, genetic damage, mutations and damage of brain, kidney and bones (WHO 2007). These heavy metals enter the food chain through natural and anthropogenic means. Industrial production, transportation, agricultural activities and mining are key emitters of heavy metals (Tangahu et al. 2011; Sardar et al. 2013), whose primary sources are use of municipal waste, sewage sludge/waste water (bio solids), manures, burning of fossil fuels, iron ores smelting during mining and use of pesticides and fertilizers (Nriagu 1979; Pendias and Pendias 1989; Wuana and Okieimen 2011).

2.2.2.2 Accumulation of heavy metals in plants

Some plants avoid uptake of heavy metals (excluders), whereas some might take up these elements through their roots, but transfer of heavy metals from roots into stems or transfer from stem to leaves might be reduced (Sekara et al. 2005). Heavy metal concentrations in different plant organs show following order in most cases: roots>stem>leaf>fruits (Andal 2016). Several factors govern the uptake of the heavy metals from the soil. Plants take up elements selectively based on the transporters in their membranes. These transporters might be selective for specific elements, e.g. Zn. Since Cd is very similar to Zn, Cd can be taken up by the plant “accidentally” (Davila et al. 2011). Besides accumulation of heavy metals through soil uptake, plants can also be contaminated with heavy metals through superficial particulate deposit. The extent of contaminated deposit depends with the levels and type of contaminants in the atmosphere, the atmospheric conditions and surface of the exposed part of the plant (Gunawardena et al. 2013). Hairy leaf surfaces accumulate more traces of heavy metals from the atmosphere than smooth surfaces, from which contaminants can be removed by washing more easily. However, the surface contaminants can be removed by wind blow or rains. Therefore, thoroughly washing of leafy vegetables before consumption may reduce or even avoid the health threat of vegetables grown in contaminated soils (Oniang'o et al. 2005). Green, leafy vegetables are the most susceptible plants to contamination by heavy metals, followed by root crops, while most fruit trees are less susceptible to heavy metal contamination (Ali and Al-Qahtani 2012). Thus, quality and safety measures of AIVs need to be looked into, to minimize ingestion of heavy metals in human dietary intakes.

2.2.2.3 Aims

The aims of the study were equivalent to those stated in chapter 2, section 2.1 under sub section 2.1.2.3, but with respect to heavy metals (Cd and Pb).

2.2.3 Materials and methods

Material and methods were as stated in chapter 2, section 2.1 under sub section 2.1.3

2.2.4 Results and discussion

Concentrations of heavy metals among different leafy vegetable species were determined. The information will be vital to nutritionist in guiding consumers on proper ways of handling vegetables before eating to avoid contamination. Extension officers and government agencies will also use the information in advising farmers and traders on good agronomic and post-harvest practices to avoid contamination with heavy metals. The effects of markets and seasons were also determined (2.2.4.2 and 2.2.4.3) in order to understand nutrient variations associated with market type and season.

2.2.4.1 Differences among species in the concentration of heavy metals

As stated earlier, the data collected from the markets was separated into data for washed samples and unwashed samples, data from open air market and supermarket and data from dry and rainy seasons. The data of washed samples were used to determine the heavy metal concentrations in vegetable species. To get the concentrations of heavy metals in individual crops, the data for seasons and markets were treated as one. In this study heavy metals considered were Cd and Pb, because of their abundance in the environment and the health effects associated with them. The presence of these elements may be a result of intensive use of artificial P fertilizer by farmers, use of untreated water and automobile movement as well as aerial collection of these heavy metal particulates.

Concentrations of Cd in the edible plant organs of the vegetables varied from a minimum of 0 to a maximum of 4.1 mg kg^{-1} leaf dry mass (Fig. 2.5a). The vegetable species varied in Cd concentration within themselves; with African nightshade having a huge variation and exotic kale a smaller one. The means were 0.37 mg Cd kg^{-1} leaf dry mass for exotic kale, 0.23 mg Cd kg^{-1} leaf dry mass for Ethiopian kale, 0.48 mg Cd kg^{-1} leaf dry mass for African nightshade, 0.24 mg Cd kg^{-1} leaf dry mass for amaranth, 0.30 mg Cd kg^{-1} leaf dry mass for cowpea and 0.30 mg Cd kg^{-1} leaf dry mass for spider plant. The mean Cd concentrations did not significantly differ among species although they varied from 0.23 mg Cd kg^{-1} leaf dry mass for Ethiopian kale to 0.48 mg Cd kg^{-1} leaf dry mass for African nightshade (Table 2.13). There were no significant differences in the Cd concentration between exotic kale and Ethiopian kale and amaranth.

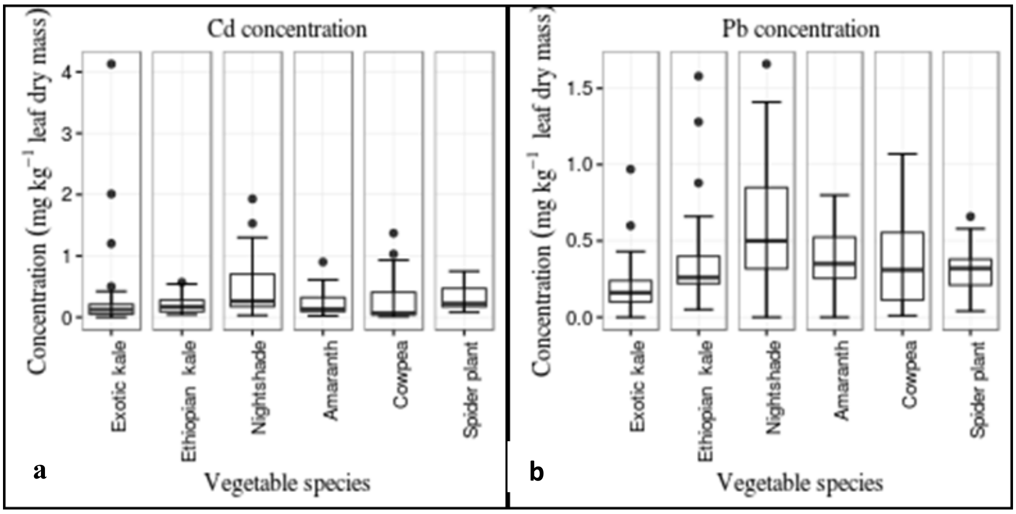

Figure 2.5: Box plots showing, minimum, first quartile, median, third quartile and maximum cadmium (a) and lead (b) concentration levels (mg kg^{-1} leaf dry mass) of samples from respective vegetable species. The dots represents (outliers) samples with extreme ranges from the rest, determined by $Q_3 + 3*IQR$ or $Q_1 - 3*IQR$ formula; where, Q_1 – first quartile, Q_3 – third quartile, IQR – interquartile range.

Table 2.14: Concentrations (mg kg^{-1} dry mass) of heavy metals in vegetable species (means ± standard deviation). Columns with same lower case letters behind the means indicate no significant differences between means ($p > 0.05$, ANOVA, LSD test)

Species	Cd	Pb
	Element concentrations (mg kg^{-1} leaf dry mass)	
Exotic kale	0.37 ± 0.83 a	0.19 ± 0.21 c
Ethiopian kale	0.23 ± 0.17 a	0.41 ± 0.41 b
African nightshade	0.48 ± 0.48 a	0.61 ± 0.45 a
Amaranth	0.24 ± 0.22 a	0.38 ± 0.22 b
Cowpea	0.30 ± 0.42 a	0.38 ± 0.32 b
Spider plant	0.30 ± 0.20 a	0.32 ± 0.17 bc

Concentrations of Pb in edible organs of the vegetables ranged from a minimum of 0 to a maximum of 1.7 mg kg^{-1} leaf dry mass (Fig. 2.5b). The range of Pb concentrations, which covered 50% of all data, was generally similar to the range of Cd concentrations (compare the extension of the boxes in Fig. 2.5a and Fig. 2.5b). The range of Pb concentrations was largest for African nightshade and, smallest for exotic kale, Ethiopian kale and spider plant. The meians were 0.19 mg Pb kg^{-1} leaf dry mass for exotic kale, 0.41 mg Pb kg^{-1} leaf dry mass for Ethiopian kale, 0.61 mg Pb kg^{-1} leaf dry mass for African nightshade, 0.38 mg Pb kg^{-1} leaf dry mass for amaranth, 0.38 mg Pb kg^{-1} leaf dry mass for cowpea and 0.32 mg Pb kg^{-1} leaf dry mass for spider plant. The mean Pb concentrations significantly differed among species (Table 2.14). The mean Pb concentration in vegetables had a minimum of 0.19 mg kg^{-1} leaf dry mass for exotic kale and maximum of 0.61 mg kg^{-1} leaf dry mass for African nightshade. Exotic kale had the significantly lowest concentration of Pb compared to all other species, except for spider plant. No significant differences were observed among Ethiopian kale, amaranth, cowpea and spider plant, but they all showed significantly higher Pb concentrations than exotic kale.

Comparing the concentrations of Cd and Pb with previous research findings, it was found that the average mean values of Ethiopian kale reported by Weldegebriel et al. (2012) were higher than our findings, although our highest value was in line with their study. The study by Nabulo et al. (2008), showed much higher concentrations of Cd and Pb in exotic kale and amaranth than found in our study. Our results were also not in line with Kananke et al. (2014), who found higher concentrations of Cd and Pb in amaranth species collected from 4 markets. Other studies have also found high concentrations of Cd and Pb in vegetables within the cities (Sharma et al. 2008b; Sharma et al. 2009). On the other hand, studies by Radwan and Salama (2006) and Fang et al. (2014), found lower concentrations of Cd and Pb in leafy vegetables.

The variation in the concentration of Cd and Pb within vegetable species can be attributed to soil factors at the production point i.e. soil pH, organic matter and soil moisture. Other factors include species type, availability of the toxic soil elements and location/sites. That was why a wide range of concentrations were seen within individual species and among vegetables species (Sharma et al. 2007). For the case of Cd, phytoavailability of total soil Cd and plant Cd uptake varies more significantly with plant species. Cd might be taken up through carriers or channels that are actually meant for necessary ions (Rangnekar et al. 2013). Since cadmium appears to be similar to other divalent ions that are necessary for cellular metabolism, e.g. Zn, low Cd to Zn ratio interferes with Cd uptake among different species (Brown et al. 1995). Cd is more likely translocated to the shoots/leaves than Pb, whose large portion is found in the roots (Fontes et al. 2014). Plants with high tolerance for Cd are likely to take up substantial amounts of the heavy metal through the plant roots and translocate it to the stems and leaves (Smolders 2001; Uraguchi et al. 2009). On the other hand, Pb has got poor solubility in water and thus plants don't take up much Pb through the roots (Tangahu et al. (2011). Studies have shown that more than a half of the amount of Pb taken up by the plants is retained in the roots and not translocated to the stem and leaves. The ability for plants to bind Pb to the roots also differs among the species (Rangnekar et al. 2013) since plant roots selectively absorb nutrients from the soil.

To save humans from contaminated food, FAO/WHO set a limit for Cd content in food to 0.2 mg per kg fresh mass and for Pb to 0.3 mg per kg fresh mass (WHO/FAO 2006). Edible plant organs exceeding these limits should not be sold and consumed. Most, if not all, of samples collected in this study were within the recommended maximum limit for Cd and Pb concentration in vegetables (Fig. 2.6), thus may be of less health concern.

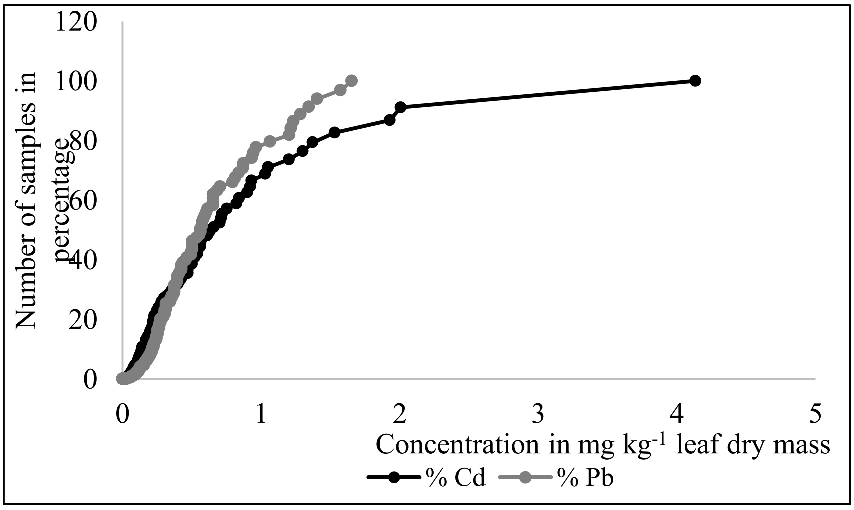

Figure 2.6: A line graph showing the distribution of samples (in percentages) based on concentrations of Cd and Pb (mg kg^{-1} leaf dry mass) measured in vegetable species.

2.2.4.2 Effects of market types on heavy metal concentrations in vegetable species

To determine the effect of market type on heavy metal concentrations in vegetable species, unwashed vegetable data were used, where wet and dry season data were combined and analysed for respective markets to get the mean concentrations for respective vegetables.

According to the study, few vegetable species showed differences in heavy metal concentrations between supermarkets and open air markets (Table 2.15). There were no differences between markets in Cd concentrations in exotic kale, Ethiopian kale, African nightshade, amaranth and cowpea. Differences in Cd concentrations between markets were seen in spider plant, where the concentration was higher in open air market (0.3 mg kg^{-1} leaf dry mass) than in supermarkets (0.19 mg kg^{-1} leaf dry mass). The study also showed that Pb concentrations in all the vegetables species were not different between the two markets.

The study is in agreement with Fang et al. (2014) who found Cd and Pb concentrations in leafy vegetables from traditional markets and supermarkets in China to be relatively equal. It was expected that supermarkets will have the highest Cd accumulations in the vegetables, while the open air markets will have high levels of Pb. Due to the probability of Cd contamination by the use of phosphate fertilizers, P concentrations of all species were analyzed, see previous chapter (Table 2.7) and higher concentrations of P were found in exotic kale and amaranth collected from super markets. The presence of Cd in vegetables found in the markets could be attributed to application of fertilizer among farms in the production of AIVs. The concentration of Cd in exotic kale from the supermarket was almost two times higher than in exotic kale from the open air market, although not significantly different. This might be related to the higher P concentrations in supermarket vegetables. The assumption is, most farmers who produce for the supermarkets are likely to give more preference to exotic kale than AIVs.

Table 2.15: Effects of market type on concentrations (mg kg^{-1} leaf dry mass) of heavy metals in vegetable species (means ± standard deviation). Rows with same lower case letters behind the means indicate no significant differences between means of a particular element ($p > 0.05$, ANOVA, unpaired T-test, n = 10 for open air markets, n = 5 for supermarkets).

Species	Cd		Pb	
	Open air market	Supermarket	Open air market	Supermarket
	Element concentrations (mg kg^{-1} leaf dry mass)			
Exotic kale	0.27 ± 0.39 a	0.49 ± 0.69 a	0.24 ± 0.21 a	0.20 ± 0.11 a
Ethiopian kale	0.17 ± 0.11 a	0.20 ± 0.15 a	0.61 ± 0.35 a	0.49 ± 0.29 a
African nightshade	0.38 ± 0.28 a	0.45 ± 0.58 a	0.87 ± 0.55 a	0.64 ± 0.44 a
Amaranth	0.26 ± 0.24 a	0.26 ± 0.27 a	0.72 ± 0.63 a	0.63 ± 0.22 a
Cowpea	0.17 ± 0.20 a	0.17 ± 0.16 a	0.82 ± 0.96 a	0.75 ± 0.55 a
Spider plant	0.30 ± 0.16 a	0.19 ± 0.01 b	1.07 ± 0.79 a	1.24 ± 0.08 a

2.2.4.3 Effects of seasons on heavy metal concentration in vegetables species

To determine the seasonal effects on heavy metal concentration in vegetable species, unwashed vegetable data was used by combining the data from both markets for a particular season and means for specified vegetables were generated. The means for vegetable species collected during the wet and dry seasons were compared by T-test analysis procedure.

Heavy metal concentrations in vegetables significantly differed between seasons (Table 2.16). Exotic kale (0.55 mg kg^{-1} leaf dry mass), African nightshade (0.54 mg kg^{-1} leaf dry mass), amaranth (0.39 mg kg^{-1} leaf dry mass) and cowpea (0.26 mg kg^{-1} leaf dry mass), had significantly higher Cd concentrations in dry season than wet season. Spider plant and Ethiopian kale did not show any seasonal effects in Cd concentration. Although differences between seasons in Pb concentration were traced in Ethiopian kale and spider plant, the seasonal effects were interchangeably. The Pb concentration in Ethiopian kale was highest in wet season (0.74 mg kg^{-1} leaf dry mass) while spider plant was highest in dry season (1.44 mg kg^{-1} leaf dry mass). The remaining vegetable species did not show significant differences between seasons.

Table 2.16: Effects of seasons on concentrations (mg kg^{-1} leaf dry mass) of heavy metals in vegetable species (means ± standard deviation). Rows with same lower case letters behind the means indicate no significant differences between means of a particular element ($p > 0.05$, ANOVA, unpaired T-test).

Species	Cd		Pb	
	Dry season	Wet season	Dry season	Wet season
	Element concentrations (mg kg^{-1} leaf dry mass)			
Exotic kale	0.55 ± 0.64 a	0.13 ± 0.17 b	0.18 ± 0.11 a	0.28 ± 0.22 a
Ethiopian kale	0.22 ± 0.14 a	0.13 ± 0.07 a	0.41 ± 0.23 b	0.74 ± 0.34 a
African nightshade	0.54 ± 0.51 a	0.28 ± 0.20 b	0.75 ± 0.55 a	0.84 ± 0.52 a
Amaranth	0.39 ± 0.30 a	0.13 ± 0.07 b	0.86 ± 0.69 a	0.53 ± 0.29 a
Cowpea	0.26 ± 0.23 a	0.07 ± 0.06 b	0.59 ± 0.70 a	1.0 ± 0.96 a
Spider plant	0.31 ± 0.14 a	0.26 ± 0.19 a	1.44 ± 0.71 a	0.57 ± 0.38 b

The present results agree with Sharma et al. (2007) who found the uptake, distribution and accumulation of Cd in *Beta vulgaris* – belongs to amaranthaceae family - higher during the summer season, whereas it accumulated more Pb in the winter season. The finding in this study differs with findings from Birnin-Yauri et al. (2011) on Pb concentrations. The study found Pb concentrations higher in leafy vegetables, sampled from fields during the rainy season. Most studies have shown that, the concentrations of Cd found in most crops are a result of plant uptake from the roots and not atmospheric contamination (Smolders 2001; Manara 2012). The high levels of Cd concentrations in the dry season were an indication that Cd can be highly absorbed by plants under high soil temperature and low soil moisture. Rieuwerts et al. 1998, found that in soils with excess water, Cd solubility was reduced, minimizing the ability of Cd to be taken up by the plants, resulting in low Cd concentrations in plants. Smolder (2001), stated that the uptake of Cd by plants is determined by soil factors and not total Cd content in the soil. Cd solubility is increased with decrease in soil pH and organic matter content (Szabela et al. 2015). The high uptake of Cd during the dry season may be attributed to high rate of transpiration due to high soil temperature (Ingwersen and Streck 2005). High decomposition rates during the dry season can release cadmium bound to organic material into the soil solution, from which it can be uptaken by plants (McGrath et al. 1994). It was expected that Pb concentration will be high in vegetables collected during the dry season (as a result of atmospheric deposition), but that was not the case. Exotic kale and spider plant never showed any effect between seasons with Cd, however they showed changes in Pb concentration under different seasons. Cowpeas showed high levels in the dry season and exotic kale in the wet season, so this cannot rule out atmospheric deposition. The accumulation of Pb in plants is basically a result of contamination, because plants take up little Pb through their roots. The presence of almost equal Pb concentrations could be an indication that most Pb contamination took place at the markets. Since markets were in one locality there was possibility of equal atmospheric deposition of contaminants throughout the year. Although, during the rainy season wash out of surface attached elements often occur (Wong 2003; Sharma 2008b), this might mainly happen at farm level.

In order to understand whether the concentrations of Cd or Pb could be a result of atmospheric contamination or not, a correlation analysis between Al and Cd was done (Fig 2.7 and 2.8). Al is an element, which is not translocated from root to shoot in most species. Thus, Al concentrations in shoots are an indicator of contamination of the shoot with dust. In dust, Al is always existing and at a higher concentration.

There was a positive relationship between the concentrations of Al and Pb (R^2 = 0,16) indicating that Pb found in vegetables species could be as a result of atmospheric contamination with soil/ dust. On the other hand, there was neither a positive nor negative relationship between the concentrations of Al and Cd in vegetable species (Figure 2.8) indicating that Cd found in vegetables was not a result of atmospheric contamination.

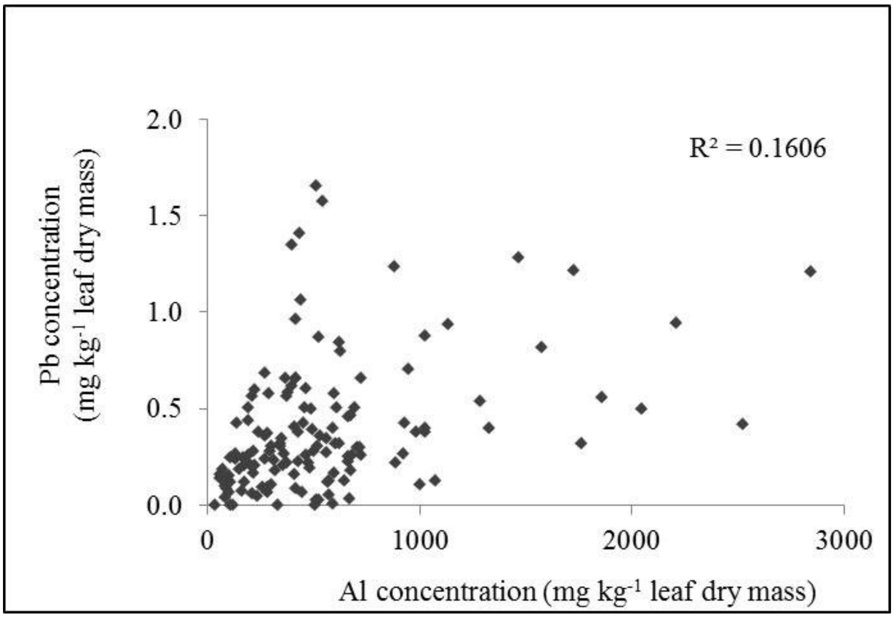

Figure 2.7: Relationship between Al and Pb concentrations (mg kg^{-1} leaf dry mass) in vegetable samples, n = 140; R^2 = coefficient of determination.

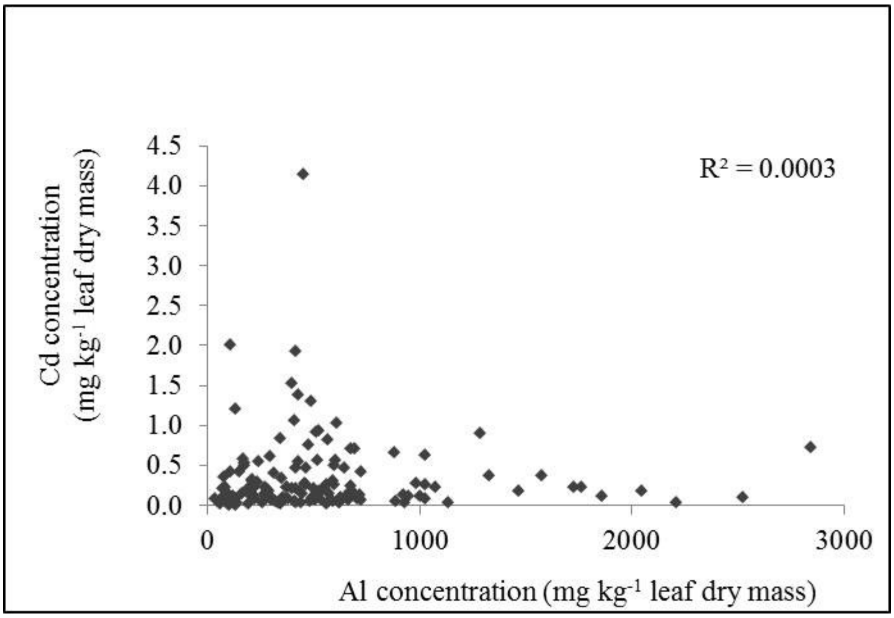

Figure 2.8: Relationship between Al and Cd concentrations (mg kg^{-1} leaf dry mass) in vegetable samples, n = 140; R^2 = coefficient of determination.

Washing of the samples was done to identify the heavy metals that can be removed and the amount likely to be eliminated in each vegetable species. To arrive at this, both, unwashed and washed data of the vegetable samples were considered and analysis was done separately.

Before analysis all the seasons and markets were combined for individual vegetable species. The respective data from unwashed samples were subtracted from washed data then divided by its respective unwashed data and multiplied by 100. Thereafter, means were generated and mean comparison among vegetable species determined.

The results showed that Cd was not reduced by washing (Table 2.17). This was indicated by negative reduction. On the other hand, washing of samples did not have any effect on the Pb concentration of exotic kale and African nightshade. Washing reduced Pb concentration to 27% in Ethiopian kale, 38% in amaranth, 42% in cowpea and 59% in spider plant.

Table 2.17: Reduction of Cd and Pb concentrations in percent due to washing vegetable species with water (Mean ± standard deviation). Columns with same lower case letters behind the means indicate no significant differences between means ($p > 0.05$, ANOVA, LSD test).

Species	Cd	Pb
	Percentage (%) elements reduction	
Exotic kale	-117 ± 57 a	-43 ± 38 b
Ethiopian kale	-92 ± 47 a	27 ± 13 ab
African nightshade	-43 ± 23 a	-2.2 ± 27 ab
Amaranth	-3.5 ± 13 a	38 ± 6 a
Cowpea	-133 ± 47 a	42 ± 7 a
Spider plant	-9.4 ± 22 a	59 ± 9 a

The reduction of Pb among Ethiopian kale, amaranth and spider plant was not significant from each other. This study is in agreement with Sattar et al. (2013), who found different washing methods to have a greater effect on Pb and Cd reduction on vegetables with tap water having a reduction of 4-6% in Cd concentration and 6 – 9% in Pb. These results agree with Sharma et al (2007) on reduction of Pb concentration in vegetables but disagree with this study's findings on effect of washing on Cd concentration. Generally, previous studies have found reduction of Cd concentrations in the leafy vegetables contrary to this study (Sing et al. 2004; Perveen et al. 2011). Leafy vegetables are known to accumulate a lot of Cd and Pb, due to environmental pollution, compared to other vegetables found in the market (Sharma 2009) and in this study high atmospheric accumulation was shown by Pb unlike Cd, although significant differences between the heavy metals were not tested. Among the vegetables, AIVs accumulated higher Pb concentrations compared to exotic kale. This could be due to low surface area to volume ratio of the kales compared to the AIVs.

Pb found in leaves of plants resulted rather from contaminated particulates of the air attached to the leaf surface, than from uptake of Pb by roots and translocation into stem and leaves, because lead is taken up rarely and thus accumulated in the roots (WHO, 2007). Whereas Cd, due to its similarity to Zn, might be taken up by Zn transporters and translocated to stems and leaves inside the plant and can therefore not be removed by washing the leaf surface like in case with Pb.

2.2.5 General discussion and recommendation

The key focus in discussion was based on the ability of washing to remove surface adhered particulates and whether the heavy metals found in the vegetables are not exceeding limits of allowed concentrations of these toxins.

Vegetable species differed in the Cd and Pb concentrations. The range of mean Cd and Pb concentrations were 0.23 - 0.48 and 0.20 - 0.61 mg kg^{-1} leaf dry mass (Table 2.14). Despite the concentration of Cd and Pb in African nightshade being higher than in other vegetable species, the concentrations were within the permissible levels allowed in leafy vegetables (WHO/FAO 2006). Sometimes vegetables may contain minimum toxic concentrations, but continuous or long term uptake might result in high accumulation of heavy metals in the body and thus leading to health complications. For that case, further calculations were needed to determine, whether the vegetables collected in this study will eventually have some health effects in the long run (Table 2.18). Using the average vegetable intake in urban and rural Kenya of 2.88 and 1.46 kg fresh mass/person/week respectively (Ruel et al. 2005), computations on weekly Cd uptake were determined based on the concentrations of Cd found in the vegetables under this study. It is recommended and care should be taken not to exceed the human tolerable weekly Cd intake of 0.175 mg and 0.406 mg Cd $week^{-1}$ $person^{-1}$ (70kg body weight) according to European food safety authority, EFSA and Joint FAO/WHO experts committee on food additives, JECFA, respectively (Clemens et al. 2012). The permissible Pb intake is 1.8 mg Pb $week^{-1}$ $person^{-1}$ (WHO 1995).

The potential dietary intake of heavy metals was calculated to determine the suitability of leafy vegetables for consumption (Table 2.1.8). The concentrations of Cd in all the vegetables were below the weekly EFSA recommendations for Cd, but were above provisional safe intake limits prescribed by agency for toxic substances and disease registry, ATSDR of 0.049 mg Cd $week^{-1}$ $person^{-1}$ (Clemens et al. 2012), in all vegetables in urban area and for African nightshade, cowpea and spider plant in rural area. On the other hand, the calculated Pb concentrations were below the weakly intake limit levels.

Table 2.18: Potential Cd and Pb intake (mg person^{-1} week^{-1}) when an average of 2.88 kg of vegetables per adult per week in urban and 1.46 kg of vegetables per adult per week in rural Kenya is comsumed. Calculations were based on the mean values across markets and growing seasons (Table 2.14) and the assumption of a dry matter content of edible organs of 11% for exotic and Ethiopian kale, 8% for African nightshade, 13% for amaranth, and 14% for cowpea and spider plant.; XK – exotic kale, EK – Ethiopian kale, AN - African nightshade, AM - amaranth, CP - cowpea and SP - spider plant.

Species	Mean urban Cd intake (2.88 kg vegetable person^{-1} week^{-1})	Mean rural Cd intake (1.46 kg vegetable person^{-1} week^{-1})	Mean urban Pb intake (2.88 kg vegetable person^{-1} week^{-1})	Mean rural Pb intake (1.46 kg vegetable person^{-1} week^{-1})
	Intake (mg Cd person^{-1} week^{-1})*		Intake (mg Pb person^{-1} week^{-1})*	
XK	0.076	0.039	0.063	0.032
EK	0.073	0.037	0.127	0.064
NS	0.111	0.056	0.141	0.071
AM	0.090	0.046	0.142	0.072
CP	0.121	0.061	0.153	0.077
SP	0.125	0.063	0.125	0.063

It was expected that vegetables drawn in the supermarkets will have high Cd concentrations, while those from the open air markets will have high concentrations of Pb. The results obtained from this study showed no differences in heavy metal concentrations in both markets apart from spider plant, which was unique by containing higher Cd concentrations in the open air market. The high concentrations of Cd in spider plant collected from open air markets cannot be attributed to the use of phosphate fertilizer by farmers as they are known to contain Cd (McLaughlin et al. 1999), since there was no indication of higher P concentration in spider plant found in the supermarkets. This means that no one is under risk of heavy metal contamination by either buying vegetables from the open air market or supermarkets, because the level of exposure and contamination was almost the same.

The high concentrations of Cd element in some vegetable species during dry season were an indication that Cd can be highly absorbed by plants under high soil temperature and low soil moisture. Thus, growing of AIVs should be avoided during the dry season in areas with high Cd concentrations in soil. Further research on moisture regimes to measure the Cd uptake by AIVs is recommended to clearly affirm this conclusion as our study has found Cd uptake in some vegetable species to be primarily dependent on seasons.

Increased contamination of Pb by atmospheric deposits was found in spider plant, cowpea and amaranth. This can be attributed to leaf size/ area and surface of the edible plant organs of the vegetables species. The hairy nature of spider plants, high canopy ability of amaranth and broad leaved cowpea trapped more dust and aerosol particulates from the atmosphere unlike exotic kale, which had a slippery surface due to waxy cuticle cover. Leaf surface contamination needs to be further looked into, to determine the extent of contamination by each species and the ability of the leaves to translocate the surface attached Pb into the internal organs of the leaves and plants in general. Washing of the vegetables eliminated traces of Pb, thus, measures for reducing contamination with Pb should be considered in the whole value chain (Shackleton et al. 2009), from field (surface cover), post-harvest handling (packaging) up to consumption (washing). For the case of Cd, effective measures should be taken at the field (e.g. selection of site, adjustment of soil pH, use of phosphate fertilizer).

Chapter 3: Phosphorus use strategies of leafy vegetables: Responses of species to different rates and forms of phosphorus supply

3.1 Abstract

Crop production for small holder farming systems of sub-Saharan Africa is often constrained by low soil contents of plant-available phosphorus (P). A better and cheaper option, rather than inorganic source of P to increase crop production, is cultivation of species with a high P efficiency. A study was conducted to measure plant responses to low P availability in soil and to determine variations among AIVs and one exotic species in their ability to use organic P and sparingly soluble P forms. Six leafy vegetables (spider plant - *Cleome gynandra*, African nightshade - *Solanum scabrum*, amaranth - *Amaranthus cruentus*, cowpea - *Vigna unguiculata*, Ethiopian kale - *Brassica carinata*, and exotic kale - *Brassica oleracea*) were cultivated under controlled conditions in pots. The substrate was amended with 49 mg P kg^{-1} soil using four different forms (highly soluble KH_2PO_4, phytate as organic P source and sparingly soluble $FePO_4$ and rock phosphate). The ability to use soluble P- forms did not differ among vegetable species, but utilization of sparingly soluble forms differed between forms of P supplied to the soil and species. All species were able to use P from phytate for biomass formation as effectively as P from KH_2PO_4. $FePO_4$ was most efficiently used by Ethiopian kale (14.4 g fresh mass $plant^{-1}$) and exotic kale (12.8 g fresh mass $plant^{-1}$), while rock P was efficiently utilized by Ethiopian kale (19.6 g fresh mass $plant^{-1}$) and cowpea (18.5 g fresh mass $plant^{-1}$). The efficiency for utilization of sparingly soluble P forms (either $FePO_4$ or rock phosphate) was low in African nightshade and spider plant. The vegetable species responded to different P treatments with significant differences in morphological root traits and changes of rhizosphere pH. In average, the overall observed rhizosphere pH across the treatments, varied from 3.4 in amaranth to 7.0 in Ethiopian kale. Under P deficiency all species increased biomass partitioning to roots. African nightshade and cowpea increased specific root length, while spider plant and amaranth increased root hair density and length in P depleted treatment. Thus, vegetable species showed large variations in root traits relevant for P acquisition and their ability to use soil P from different sparingly soluble P forms. For that case, species and site-specific properties have to be considered to choose optimal plant species for certain fields containing known P-forms in soil.

Key words: P acquisition, rock phosphate, phytate, rhizosphere pH, root morphology

3.2 Introduction

3.2.1 Soil phosphorus pools and their availability to plants

Low P availability in agricultural soils is a major constraint to better crop growth, quality and yield. P is among the most quantitatively needed minerals, besides N and K, needed by plants to perform processes such as respiration, photosynthesis, energy generation, N_2-fixation, carbohydrate metabolism, glycolysis, respiration, enzyme activation/inactivation and redox reactions and is part of several plant structures (e.g. phospholipids, Vance et al. 2003; Balemi and Negisho 2012). Sufficient availability of P for plants promotes early maturity, enables far-reaching, vigorous root development and support resistance to diseases among plants. P deficiency may result in reduced growth rate, increased production and secretion of phosphatases, exudation of organic acids, increased root growth and modified root architecture, increased root surface area and increased expression of pi transporters (Vance et al. 2003).

Plants take up P from the soil rhizosphere within the reach of the roots. Soil P exists in two main chemical forms including inorganic P (Pi) and organic P (Po). Po tend to be high in most Alfisols soils of tropical Africa constituting about 30 - 50% of total P (Reddy et al. 2005), while Pi in the soil ranges from 35 - 70% of the total P. Primary P minerals, like variscite, apatites or strengite are not readily available to plants as a result of slow release of available P by weathering processes, although direct application of apatites (Rock P) has proven to be effective for crops grown in acidic soils (Shen et al. 2011). Three main pools of P exist in soil, namely solution P, active P and fixed P. Solution P comprises mostly inorganic P and small amounts of organic P and represents the pool from which plants take up P. Active P is a solid phase of P easily released to the soil solution, thus remaining as a source of available P to crops. Active P is made up of organic P that is readily mineralized and inorganic phosphate that is adsorbed to Ca and Al to form soluble solid P. Fixed P contains insoluble inorganic P and organic P resilient to mineralization. This pool is no longer available to plants and may exist in the soil for years, thus having little impact on P soil fertility (Balemi and Negisho 2012). At low pH and presence of Fe/Al, P is fixed as Fe-/Al-P, at high pH and presence of Ca, P is fixed as Ca-P. Soil solution typically contains around 2 µM Pi, while plants show concentrations of Pi from 5 to 20 mM (Bieleski 1973). The optimal pH for effective uptake of inorganic P ranges between 4.5 and 5.0 (Raghothama 1999; Vance et al. 2003), highest uptake rates are at pH 5.0 - 6.0, where $H_2PO_4^-$ dominates (Schachtman et al. 1998). In mineral soils larger portions of P ions get to plant roots through diffusion. Only small portion of phosphate ions (1-5% of plant demand) reach the root surface via mass flow (Balemi and Negisho 2012). Two main factors affect plant/crop P content, one factor is the uptake ability by the crop and the other factor is phosphorus availability in the soil solution (Beegle and Durst 2002). Thus, in most food crops, the P concentration ranges from 0.05 to 0.50% dry weight (Vance et al. 2003).

Understanding of P dynamics and movement from soil to plant calls for improving P-uptake efficiency and proper phosphorus management thus reducing the need for high input of inorganic fertilizer.

3.2.2 Plant traits relevant to soil phosphorus acquisition

Plants take up P from the soil solution in form of anions. The ability to take up P is determined by the plant species and the available soluble P in the rhizosphere (Hammond et al. 2009). Variation of P uptake in plants is majorly determined by physiological and biochemical factors; P transporters, root morphology (specific root length, root diameter, root hairs), the mycorrhiza symbiosis mechanism and rhizosphere processes (or-ganic acid release, pH adjustment, phosphatase activity) among other traits (Gahoonia1 and Nielsen 1996).

Deficiency of plant available P in the soil has been a worldwide problem to many agricultural crops. Reducing P demands can enhance phosphorus sustainability in crop production systems. Under low supply of P, plants have adopted strategies to improve P acquisition and growth. Some of these strategies include root foraging to improve spatial soil exploitation, P mining strategies to enhance desorption, solubilization or mineralization of soil P and improving internal P utilization efficiency (Richardson et al. 2011). Plant species behave differently towards the four forms of P existent in the soil (Table 3.1). Thus, different plant characteristics will emerge.

Table 3.1: Plant traits related to P efficiency for different forms of soil P.

Plant trait/rhizosphere characteristic	Effective for
Internal use efficiency (biomass formation per unit plant P)	All P forms
Root surface (e.g., root hairs, specific root length)	All P forms
Rhizosphere phytase activity	Organic P
Low rhizosphere pH, release of carboxylates (e.g., citrate)	Calcium-P
High rhizosphere pH, release of carboxylates (e.g., citrate)	Fe-P, Al-P

3.2.3 Soil phosphorus status in small holder farming systems in sSA

Phosphorus depletion in Rwanda, Ethiopia and Kenya soils is at an alarming state, where annual depletion rates go beyond 6.6 kg P ha^{-1} (Smaling et al. 1997). The main causes of P deficiency vary from intensive cultivation without adequate nutrient replenishment, farming systems, to water erosion. Besides P losses, soils in East Africa, which comprise acrisols, ferralsols and nitisols are prone to fixing P in the soils due to their acidic nature. Wealth status of farmers has contributed immensely to variability of mineral fertilizer use between the rich and the poor (Zingore et al. 2007). In most cases poor farmers have found mineral fertilizers being inaccessible and unaffordable leading to less or no application of fertilizers, resulting in poor crop growth and yield.

Crop growth and yield has been significantly improved by application of P fertilizer, which has caused increased environmental risks, adsorption of P leading to high accumulation of P in the soil and low P utilization efficiency (Li et al. 2011). Farmers mainly use P fertilizers and organic P in form of animal manure and compost. In the process, only 10 - 25% of the applied P is utilized by the plants, the rest is adsorbed by the soil (about 70-90%) and transformed into a form that is not available to plants (Wang et al 2015; Bai et al. 2016). This implies that much P fertilizer is needed to achieve a higher yield, thus become too costly for smallholder farming systems.

To enhance food productivity among the resource poor farmers, reliable and reasonably priced mineral P fertilizer is needed, which has been a challenge in most countries. Thus, the best option is to identify crop varieties, with ability to withstand P poor soils and produce an equivalent high yields (George et al. 2016). The study and selection of species with great P efficiency will result in high use of soil P, increased P fertilizer uptake efficiency leading to reduced fertilizer requirements (Shujie and Yunfa 2011). Hence there is need to protect the environment and improve yield, since vegetables demand more P fertilizers as compared to other crops. This is why this study was conducted to measure plant responses to low P availability in soil and to determine variations among AIVs in their ability to use organic P and sparingly soluble P forms in comparison to soluble P. Currently, there is no available data on the utilization of P forms by AIVs and their interaction effects on P deficient soils.

3.2.4 Aims

In this study, the accumulation of P in plant species and internal P utilization efficiency was quantified under different P treatments in order to examine, which vegetable species can perform well under the investigated P forms. The information obtained will act as a guideline to farmers, which vegetables species to choose for production as a function of soil type found on their farm/field. Specifically, we were interested to know whether AIVs can yield more, when grown in soils with different P forms, than exotic kale. It is known that different crop species have different ability to take up different forms of P in the soil. This is based on the type of species and ability of plant roots to exploit soil and modify the rhizosphere in order to obtain sparingly and highly soluble phosphorus from all directions within the soil rhizosphere. Since the vegetable species investigated were drawn from different plant families, there are possibilities for some of them to have high affinity for either organic, sparingly or highly soluble P. Through yield, we shall be able to determine which species can yield more with less P uptake and vice versa.

It is known that plants adapt to P limitations by changing root length, fine root density, rhizosphere pH and/or biomass partitioning, as mentioned above. So these parameters were analyzed in all AIVs and exotic kale. The different forms of P considered for the analysis were KH_2PO_4 representing the mineral/ highly soluble phosphorus form, phytate representing the organic P form and Fe-P as well as rock phosphate representing adsorbed P in acidic soils and calcareous soils, respectively.

3.3 Material and methods

3.3.1 Study area

The experiment was conducted in the glass house at Humboldt University, Research Institute Albrecht Thaer Weg – Dahlem in the south-west of Berlin. The study was done during the autumn period (14th October to 26th November 2015). The temperatures in the glass house were regulated between 20 - 25°C during the day and between 18 - 20°C at night using automated greenhouse control system. Light from electrical bulbs were used to provide artificial lighting to enhance plant light hours and radiation between 0530 – 0800 hrs and 1630 – 1900 hrs. This was to ensure that the conditions are in conformity with the sSA mean temperature and light durations.

3.3.2 Experimental design

The experiment was set up as a two factorial experiment with six species (spider plant - *Cleome gynandra,* cowpea - *Vigna unguiculata,* amaranth - *Amaranthus cruentus*, African nightshade - *Solanum scabrum*, Ethiopian kale - *Brassica carinata* and exotic kale - *Brassica oleracea acephala*) and five P treatments (49 mg P per pot in the form of potassium phosphate - KH_2PO_4, phytate, iron phosphate - $FePO_4$, rock phosphate - $CaPO_4$ and no P). Each treatment comprised four replicates, resulting in a total of 120 pots (Fig.

3.1). Nutrients were added to each pot in the quantity indicated in Table 3.2. Vegetable seeds planted were purely bred and supplied by the World Vegetable Centre (AVRDC) located in Tanzania. During pot set up, 49 kg P pot^{-1} (Table 3.2) was mixed with the substrate as described in section 3.3.3, except for the -P pots. A complete randomization of treatments within each replicate and randomization of species among replicates were done. This was achieved by labeling species and their treatments, then a table of random numbers was used to select from the labelled subjects.

Figure 3.1: Arrangement of pots in the glass house; where A - a photo showing the manner in which pots with growing vegetables were arrangement in one of the four replicates, B – a photo showing the arrangement of pots in the four replicates with different vegetable species arranged horizontally and treatments arranged vertically (n=120).

Table 3.2: Macro nutrients applied at the start of the experiment in mg per pot within the different P treatments and levels (n = 120).

Treatment	P mg pot^{-1}	N mg pot^{-1}	K mg pot^{-1}	Ca mg pot^{-1}	Mg mg pot^{-1}	S mg pot^{-1}
without P	0	61	62	82	18	53
KH_2PO_4	49	61	62	82	18	53
Rock P	49	61	62	82	18	53
$FePO_4$	49	61	62	82	18	53
Phytate	49	61	62	82	18	53

3.3.3 Substrate and pots

Before planting, sand granules were thoroughly washed and rinsed several times with distilled water to wash out adsorbed P on the surfaces of sand and any other minerals that may be present in sand. The washed sand was air-dried for three days. After drying, sand was mixed with non-fertilized soil, whose chemical composition was known, in the ratio of 60:1 (1.2 kg sand and 20 g soil) during individual pot filling. The purpose of the soil was to provide soil microorganisms. Soil bulk density was adjusted to 1.3 g/cm^3in each pot.

3.3.4 Planting, watering and general plant management

Due to different speed in germination and biomass production at the start of the growth period, planting of the different species was done on different days, starting with the species, which develops slowest at the beginning of our experiment. Ten seeds of Amaranth were planted in each pot on the first day (14th October, 2015), followed by spider plant and African nightshade (both 10 seeds/pot) on 15th October. The kales (Ethiopian and exotic) were planted on 16th October, 2015 and cowpea was the last species to be planted on 19th October with four seeds planted in each pot. The crops differed in crop emergences with Cowpea taking three days to emerge whereas the rest took at least five days to fully emerge from the soil.

First thinning was done on 27th October, where only four plants were left on each pot containing spider plant, amaranth, African nightshade, Ethiopian and exotic kale. For cowpeas, two plants were left per pot. The second thinning was done six days after first thinning, whereby, only one plant was left, for all the species, to grow until harvesting.

Additional 0.25ml of 0.5M ammonium nitrate were applied sequential to all the pots on 28th October, 4th November and daily starting from 9th November. Watering of the pots up to 80% water holding capacity (equivalent to approximately 236 ml) was done daily. Before watering, the pots were placed on a weighing balance to determine the amount of water lost as a result of evapotranspiration. The amount was recorded for later tabulation and comparison. After noting the amount of water lost, each pot was filled up to the required weight. Monitoring of water levels was done to ensure that it did not drop below 40% water holding capacity. The times of watering doubled when nearing harvesting time. Water loss was high (especially for cowpeas and African nightshade), thus watering was done in the morning and evening. The second and last reapplication of N, K, Mg, S and micronutrients was done on 17th November with same rates as shown in table 3.2.

3.3.5 Measurement of biomass

All the vegetable species were harvested on 26th November 2015. Plant height was recorded and vegetables were harvested by cutting the stem 5 cm above the soil and the above ground biomass fresh weight was recorded and afterwards packed in a bag and oven-dried at 70°C for two days.

The rhizosphere pH was determined by adding 100 ml more water than needed to reach 100% of water holding capacity. The leachates were collected in a beaker and pH measurements were done immediately using a pH meter. The recorded pH readings were compared with the initial soil pH (6.0) taken before planting. Thereafter, the substrates in each pot were carefully removed and a subsample of each substrate was air-dried and later taken for analysis.

All the roots in each pot were carefully removed by soaking them in distilled water in order to loosen the attached soil and facilitating to remove the roots. The roots were immediately placed in a beaker containing water to avoid any mineral loss or drying. Root samples were used to determine root length, root hair density, root diameter and fresh weight. After root characterization, root samples were dried at 70°C. Dried samples (root and shoot) were crushed in a ball mill (Retsch type MM2) for approximately two minutes into powder form. The powder was then packed in small labeled plastic bottles for chemical analysis.

3.3.6 Analysis of morphological root traits

The roots were taken to the laboratory for further preparations. First, whole roots for each species planted under different phosphorus forms were mounted on the wall and a photo of the structure was taken. Sub samples of the roots from KH_2PO_4, rock P and without P treatments were picked for scanning. Root fly (Birchfield 2008) and Gimp 2.8 software (Matti 1995) were used to determine root length and diameter from the scans. The roots from KH_2PO_4, rock P and without P treatments, were observed through high level concave lenses (1 or 2 mm diameter) microscope to analyze the number and distribution of root hairs. Root hair density was determined by counting the number of root hairs taken from ten different roots of the same treatment and divided with the lens diameter.

3.3.7 Chemical analysis

250 mg of each powdered sample was weighed in a glass bottle and burned in a furnace chamber at 500^o C for 12 hours. The samples were cooled and 2.5 ml of nitric acid (1:3 diluted 65% HNO_3) was added, then placed in a burner to evaporate the acid. Again samples were placed in a furnace chamber at 500^o C for 2 hours. The samples were cooled and the residue dissolved in 2.5 ml of 1:3 diluted 37% hydrochloric acid. The mixture was then transferred to a 25 ml flask and double distilled water was added up to the mark. The liquid was then filtered into a clean well labeled plastic bottle using size 42 filter paper. The filtered liquid was subjected to Inductively Coupled Plasma with Optical Emission Spectroscopy (ICP – OES) analyzer for macro and trace element determination in the vegetable sample.

3.3.8 Calculation of apparent, relative and internal phosphorus use efficiency

Apparent values were calculated by taking the values of P concentration or dry weight values or root to shoot ratio obtained from treatment without P and subtract them from KH_2PO_4, phytate, rock P and $FePO_4$treatments.

Relative values of P concentration, dry weight and root to shoot ratios were calculated based on the KH_2PO_4 treatment; all the values for this treatment were treated to be 100%, thus relative value was equal to value from either phytate, rock P or $FePO_4$ dived by values from KH_2PO_4 of the respective species multiplied by 100. Internal phosphorus use efficiency was equal to dry mass produced by a particular species divided by phosphorus uptake of the same species.

3.3.9 Statistical analysis

Data on plant biomass, plant P concentrations, root morphology and rhizosphere leachate pH, were analysed using R statistical software for means and standard deviation. The residuals were tested for normality using q-q plots, histogram and density mean. Homogeneity of variances was tested using Bartlett's test for equal variance. ANOVA was performed to determine statistical differences ($p < 0.05$) among treatments and species. A further post hoc LSD test for mean separation was also done.

3. 4 Results and discussion

In this section we first (3.4.1) consider the effects of the P treatments (different P forms) on shoot fresh mass. Shoot fresh mass is sold by the farmers, and thus, from the farmers' point of view is presumably the most important parameter for assessing P-form effects, and answering the question if species differ in their yielding ability on fields in which different forms of P are prevailing. Second (3.4.2), we consider the effects of the P treatments on total plant biomass. About 45% of the plant biomass is consisting of organic C. Thus, from the agronomists´ and crop physiologists´ point of view, total plant biomass is an important parameter for assessing the ability of species to use different P forms in soil for C assimilation. Differences among species in the ability to use various external P forms may result from uptake efficiency, and internal P utilization efficiency. To assess the role of uptake and internal P utilization efficiency for explaining species-specific P form effects, we then consider effects of the P treatments on P accumulation (3.4.3), and biomass production per unit of accumulated P (3.4.4). In response to different P supply, plants may change biomass allocation between organs responsible for acquisition of soil resources (i.e. roots) and organs responsible for acquisition of light and CO_2 (i.e. shoots). Another class of responses is change of morphological and physiological root characteristics. All these changes may lead to increased plant fitness, and thus, may be regarded as plant adaptation to a specific rate and form of P supply. In the last section (3.4.5) we present data on species-specific responses to assess, whether these responses may explain species differences in P use efficiency, and the use of different P forms.

3.4.1 Effect of P treatments on yield (shoot fresh mass)

Phosphorus treatment effects became visible about two weeks after plant emergence. The most obvious visible P treatment effects were differences in total plant growth and leaf size, whereas typical symptoms of P deficiency described in textbooks, such as senescence of basal leaves, dark green colour and reddish marbling of non-senescent leaves and stems were nearly absent (Fig. 3.2).

At harvest, the shoot fresh mass was significantly influenced by species and P treatment, whereby the P treatment effect was dependent on species (significant interaction species x P treatment) (Table 3.3). In plants supplied with readily soluble inorganic P (KH_2PO_4), shoot fresh mass decreased in the order cowpea > Ethiopian kale and African nightshade > exotic kale > spider plant and amaranth.

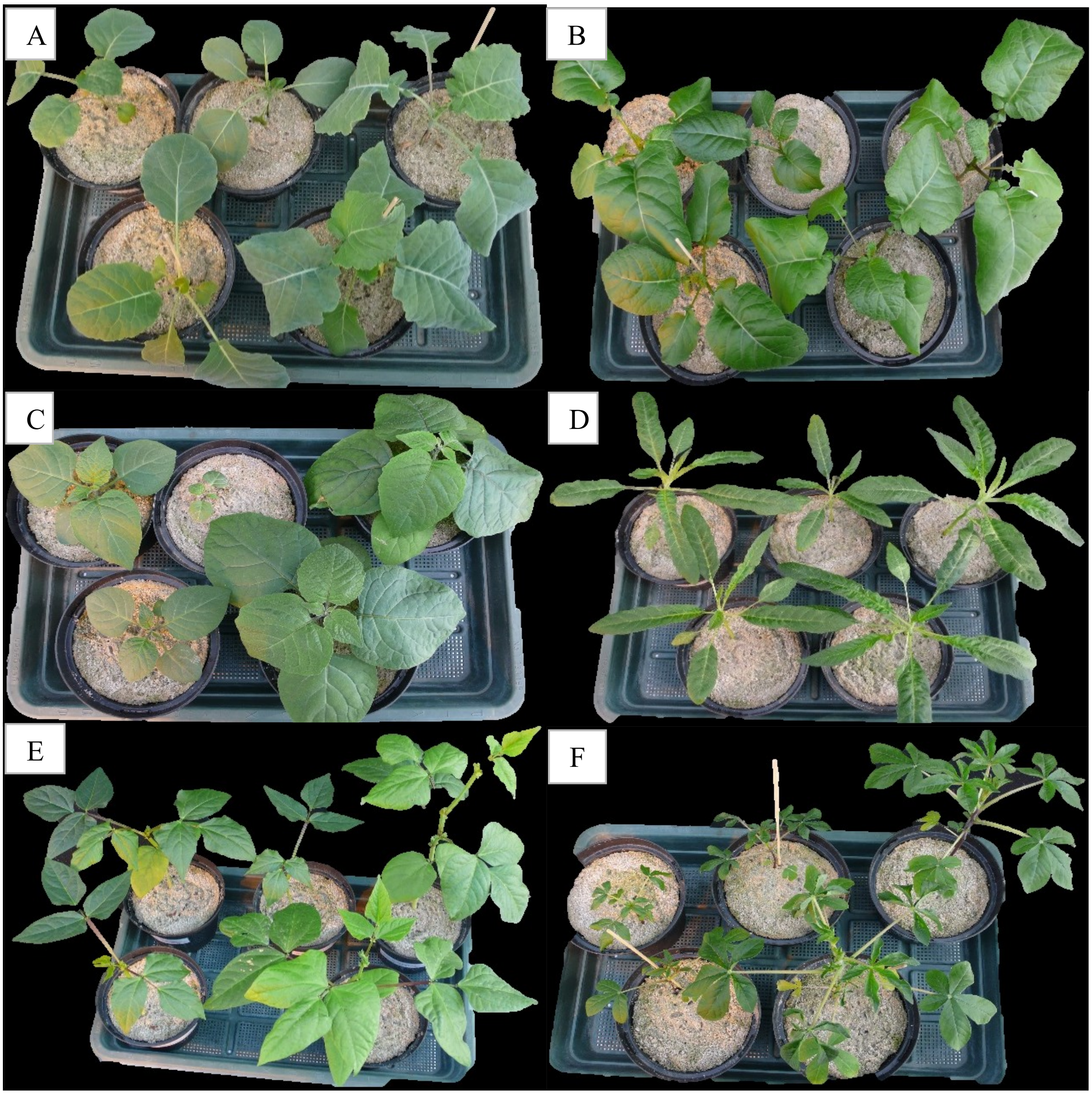

Figure 3.2: Appearance of vegetable species 4 weeks after sowing. Each tray represent a particular species with vegetables in the pots arranged systematically according to the P forms (treatments) used. In each tray, starting from the top left moving in clockwise direction, the treatments are; rock P, without P, phytate, KH_2P0_4 and $FePO_4$, where A – exotic kale, B - Ethiopian kale, C – African nightshade, D – amaranth, E – cowpea and F – spider plant.

This show that under conditions, in which P was not limiting for plant growth, shoot fresh mass differed among species. Possible reasons for growth differences under these conditions include the rate of emergence and infestation with seed-borne diseases (Bachelor-thesis Hoeppner).

Shoot fresh mass of plants supplied with organic P in the form of phytate did not significantly differ from that of plants supplied with KH_2P0_4. This indicates that phytate was a good source of P supply for all species (Table 3.4). This is in agreement with studies on other crop species (Abel 2011). It is well documented, that phytases derived from plants and/or microorganisms can release orthophosphate P from phytate, and thus make phytate-P available for plants (Richardson et al. 2011).

Table 3.3: Effect of P-treatment on shoot fresh mass (g $plant^{-1}$) of different vegetable species (mean ± standard deviation); where XK: exotic kale, EK: Ethiopian kale, NS: African nightshade, AM: amaranth, CP: cowpea, SP: spider plant. Columns with same lower case letters behind the means indicate no significant differences between means of vegetable species, rows with same upper case letters behind the means indicate no significant differences between means of treatments ($p > 0.05$, ANOVA, LSD test, $n = 4$).

Species	P-treatment				
	KH_2PO_4	Phytate	Rock P	$FePO_4$	Without P
	Shoot fresh mass (g $plant^{-1}$)				
XK	18.3 ± 2.0 cA	17.1 ± 1.0 cdA	12.6 ± 3.0 bB	12.8 ± 1.2 aB	6.0 ± 0.9 aC
EK	21.9 ± 1.9 bA	20.8 ± 0.5 bAB	19.6 ± 0.3 aB	14.4 ± 0.5 aC	6.4 ± 0.7 aD
NS	21.4 ± 0.8 bA	18.5 ± 2.8 bcA	7.2 ± 3.7 cB	3.9 ± 0.7 dC	1.0 ± 0.5 bC
AM	13.1 ± 2.2 dA	14.8 ± 0.7 dA	12.6 ± 1.5 bA	6.4 ± 2.2 cB	2.4 ± 0.9 bC
CP	25.4 ± 1.9 aA	25.1 ± 1.9 aA	18.5 ± 1.0 aB	9.5 ± 1.3 bC	5.7 ± 2.1 aD
SP	14.5 ± 1.4 dA	14.8 ± 3.3 dA	2.5 ± 0.8 dB	2.1 ± 0.4 dB	1.1 ± 0.9 bB

Shoot fresh mass of plants supplied with rock P was significantly lower than that of plants supplied with KH_2PO_4 for all species except amaranth. However, there were marked differences among species in the extent of growth reduction, indicating that the suitability of rock P as fertilizer source was strongly dependent on the specific species (Table 3.4). Whereas growth reduction of plants supplied with rock P in comparison to KH_2PO_4 was only 11% in Ethiopian kale, growth was reduced by about 30% in cowpea and exotic kale, by 66% in African nightshade, and by 83% in spider plant. Rock P is a comparably cheap P fertilizer, which is produced in several African countries. Rock P contains mainly poorly water soluble calcium phosphate.

Shoot fresh mass of plants supplied with $FePO_4$ varied between 2.1 g for spider plant and 14.4 g for Ethiopian kale, and thus, was significantly lower for all species than shoot fresh mass of plants supplied with KH_2PO_4. Again, there were marked differences among species in the extent of growth reduction indicating that the suitability of $FePO_4$ as fertilizer source was strongly dependent on the specific species (Table 3.4). Whereas growth reduction of plants supplied with $FePO_4$ in comparison to KH_2PO_4 was only 32% for exotic kale and 34% for Ethiopian kale, growth was reduced by 51% in amaranth, by 63% in cowpea, by 82% in African nightshade, and by 86% in spider plant.

The shoot fresh mass of plants cultured in substrate without P supply varied between 1 g for African nightshade and 6.4 g for Ethiopian kale. Shoot fresh mass was significantly higher in Ethiopian and exotic kales and cowpea, than for amaranth, and for amaranth, shoot fresh mass was significantly higher than for African nightshade and spider plant. The P content per seed of cowpea (700 µg P) and the kales (31 µg P in exotic, and 43 µg P in Ethiopian kale) was substantially larger than in spider plant (10 µg), African nightshade (9 µg) and amaranth (3 µg). Larger seed P content might have allowed better initial root growth, and thus better spatial exploitation of the soil volume, and consequently, more efficient use of the small plant available soil P pool in non-fertilized pots for shoot growth (Raboy 2001; Zhu and Smith 2001).

Based on the shoot fresh mass reduction in comparison to KH_2PO_4 supply, phytate was a good source for all species, whereas the suitability of other inorganic P sources for shoot fresh mass growth strongly differed among species (Table 3.4). For spider plant, shoot fresh mass of plants supplied with $FePO_4$ or rock P did not significantly differ from shoot fresh mass of plants without P amendment, indicating that $FePO_4$ and rock P are extremely poor P sources for this species. For African nightshade, the shoot fresh mass of plants supplied with $FePO_4$ did not significantly differ from shoot fresh mass of plants without P amendment, whereas shoot fresh mass of plants supplied with rock P was significantly higher than shoot fresh mass of plants without P amendment. Nevertheless, the shoot fresh mass with rock P was substantially (more than 50%) lower, than with phytate and KH_2PO_4, indicating that rock P is a poor P source for African nightshade. For cowpea, shoot fresh mass of plants supplied with $FePO_4$ was substantially reduced, whereas shoot fresh mass of plants supplied with rock P was only moderately lower than shoot fresh mass of plants supplied with KH_2PO_4 indicating, that for this species $FePO_4$ is a poor, and rock P a fairly good P source. Based on the shoot fresh mass reduction in comparison to KH_2PO_4 supply, rock P and $FePO_4$ were fairly good P sources for exotic kale. For amaranth, rock P was a good P source, whereas $FePO_4$ was a poor

P source. For Ethiopian kale all P forms were good or fairly good sources for shoot fresh mass growth.

Table 3.4: Suitability of different P sources for yield production (shoot fresh mass) in various AIVs; +++ about same (± 20%) yield effect as KH_2PO_4; ++ yield reduction 20-50% in comparison to KH_2PO_4; + yield reduction >50% in comparison to KH_2PO_4 but significantly higher yield than without P amendment; - no significant increase in yield in comparison with no P amendment ($p > 0.05$, ANOVA, LSD test, n = 4).

Species	P source		
	Phytate	Rock P	$FePO_4$
Spider plant	+++	-	-
African nightshade	+++	+	-
Cowpea	+++	++	+
Exotic kale	+++	++	++
Amaranth	+++	+++	+
Ethiopian kale	+++	+++	++

3.4.2 Effect of P treatments on total biomass

We assessed the effect of P treatments on the total biomass, which is made up of shoot and root biomass in order to assess the ability of plant species to assimilate carbon. The total dry mass (total biomass) was significantly influenced by the plant species and P treatment (Table 3.5). The performance and trend of vegetable's total dry mass was somehow similar to the yield (shoot fresh mass).

In the plants supplied with readily soluble inorganic P (KH_2PO_4), total dry mass decreased in the order cowpea and African nightshade > exotic kale and Ethiopian kale > amaranth and spider plant. This also shows that under conditions in which P was not limiting for plant growth, total dry mass differed among species. Total dry mass of plants supplied with organic P in the form of phytate did not significantly differ from that of plants supplied with KH_2P0_4 apart from amaranth, which showed higher biomass with phytate supply than with KH_2PO_4supply.

Total dry mass of plants supplied with rock P was significantly lower than that of plants supplied with KH_2PO_4 for all species except for Ethiopian kale. For Ethiopian kale, total biomass did not significantly differ between rock P supply and supply of phytate or KH_2PO_4.

Table 3.5: Effect of P-treatment on total dry mass (g plant $^{-1}$) of different vegetable species (mean ± standard deviation). Columns with same lower case letters behind the means indicate no significant differences among means of vegetable species, rows with same upper case letters behind the means indicate no significant differences among means of treatments ($p > 0.05$, ANOVA, LSD test, n = 4); XK: exotic kale, EK: Ethiopian kale, NS: African nightshade, AM: amaranth, CP: cowpea, SP: spider plant.

Species	P-treatment				
	KH_2PO_4	Phytate	Rock P	$FePO_4$	Without P
			Total dry mass (g plant^{-1})		
XK	3.0 ± 0.3 bA	3.1 ± 0.4 bA	1.9 ± 0.5 bB	1.8 ± 0.2 abB	1.0 ± 0.2 aC
EK	3.5 ± 0.1 bA	3.3 ± 0.3 bA	3.2 ± 0.5 aA	2.1 ± 0.1 aB	1.1 ± 0.1 aC
NS	4.3 ± 0.5 aA	4.3 ± 0.5 aA	1.0 ± 0.6 cB	0.6 ± 0.2 dBC	0.2 ± 0.1bC
AM	2.2 ± 0.5 cB	2.8 ± 0.2 bcA	1.8 ± 0.2 bB	1.2 ± 0.4 cC	0.5 ± 0.2 bD
CP	4.2 ± 0.3 aA	4.1 ± 0.3 aA	3.3 ± 0.5 aB	1.7 ± 0.3 bC	1.0 ± 0.3 aD
SP	1.9 ± 0.3 cA	2.2 ± 0.7 cA	0.4 ± 0.1 cB	0.4 ± 0.1 dB	0.2 ± 0.1 bB

However, there were marked differences among species with Ethiopian kale and cowpea having the highest total dry mass. Total dry mass of plants supplied with $FePO_4$ was significantly lower than that supplied with KH_2PO_4 for all the species. Total dry biomass production in plants supplied with $FePO_4$ ranged from 2.1 g plant^{-1} for Ethiopian kale to 0.4 g plant^{-1} for spider plant. Considering poorly soluble inorganic P forms, total dry mass of exotic kale, amaranth and cowpea supplied with $FePO_4$ was significantly lower than that supplied with rock P. Total dry mass of plants not supplied with P were significantly lower than those plants, which were supplied with the different P treatments apart from spider plant and African nightshade. Exotic kale, Ethiopian kale and cowpea showed similar biomasses and grew a little better in unfertilized soils than African nightshade, amaranth and spider plant, which had a lower biomass.

Most studies have shown reduced biomass (shoot biomass) production in plant species, when grown under P deficient soils compared to soils treated with highly soluble P (Pearse et al. 2006; Hammond et al. 2009; Wang et al 2010; Shen et al. 2011; Rose et al. 2013; Lambers et al. 2015). At this state, P is inadequate to plants and thus plants are unable to efficiently undertake their physiological processes leading to low plant biomass accumulation. Also the study by Liang et al (2009) showed increased dry matter yield of amaranth with increasing P rates.

Generally, cowpea performed well, while spider plant performed poorly in all the P treatments. On the other hand, Ethiopian kale produced the highest dry mass in sparingly soluble P treatment and in soils without P compared to other species. This implies that cowpea and Ethiopian kale are likely to have properties superior in P use efficiency compared to the other species, which will be analyzed further in this study. The ability for cowpea to produce well in all the P treatments makes the plant suitable for growth in all types of soils composed with different P pools. A study by Ansah et al. (2016), found out that a reasonable number of cowpea genotypes is able to respond and produce well under low P and sufficient P media, thus is in agreement with our study. Also Alkama et al (2008), found cowpea to grow better in P deficient soils compared to soy bean and common beans. Crop species that respond well to presence or absence of P are the most desirable. Our findings are not in agreement with Oladeji et al. (2006), who found no differences between yields of cowpea in rock P and without P treatments.

3.4.3 Effect of P treatments on shoot and root P concentrations and plant P accumulation

In the shoot, P concentration was influenced by P forms and the plant species (Table 3.6). Shoot P concentration in plants supplied with KH_2PO_4 was significantly higher than in those plants supplied with other forms of P. This was due to the high solubility of KH_2PO_4. Species utilized KH_2PO_4 differently, varying in P concentrations from 10 mg per kg dry mass for spider plant and 4.9 mg P per kg dry mass for African nightshade. Acros species the shoot P concentration of plants supplied with phytate was significantly lower than of those supplied with KH_2P0_4. Species supplied with rock P had significantly lower shoot P concentrations than those in KH_2PO_4-treatment. Rock P was efficiently acquired by African nightshade, amaranth, Ethiopian kale and exotic kale showing the same concentration of P in the shoot as species supplied with phytate. Amaranth had the highest concentration (4.9 g kg^{-1} dry mass), when rock P was supplied, while cowpea was least in P concentration (2.7 g kg^{-1} dry mass). Shoot P concentration of all species supplied with $FePO_4$ was lower than that supplied with rock P, apart from spider plant. Amaranth and Ethiopian kale had significantly lower shoot P concentrations when not supplied with P compared to $FePO_4$ and other P treatments. Spider plant could take up minimal amounts of P into the shoot, when grown under $FePO_4$, rock P and without P. The differences in shoot P concentration among species supplied with different P forms contributed to differences in shoot biomass production among species.

On the other hand, shoot P concentrations did not strongly vary among the P treatments in African nightshade, whereas for spider plant concentrations strongly varied. This is relevant under two points of view. First, for assessment of the P nutritional status of plants through chemical plant analysis, it is desirable that plant P concentration strongly responds to alteration of the P status in the growth media. As such, spider plant is better suited for plant analysis than African nightshade. Second, for agronomic P efficiency (saving P fertilizer) it is desirable that leafy vegetables do not accumulate high internal P reserves under conditions of high soil P availability, because high concentration leads to high P transfer to the market. As such, spider plant is worse than African nightshade.

Table 3.6: Effect of P-treatment on shoot P concentration (g kg^{-1} dry mass) in different species of AIVs (mean ± standard deviation). Mean values marked with same small letters within a column indicate no significant differences among the species, mean values marked with same capital letters within a row indicate no significant differences among different P-treatments ($p > 0.05$, ANOVA, LSD test, $n = 4$); XK: exotic kale, EK: Ethiopian kale, NS: African nightshade, AM: amaranth, CP: cowpea, SP: spider plant.

Species	P-treatment				
	KH_2PO_4	Phytate	Rock P	$FePO_4$	Without P
	Phosphorus concentrations in the shoot (g kg^{-1}dry mass)				
XK	6.8 ± 1.2 cA	3.3 ± 0.4 bB	3.8 ± 0.9 bcB	2.7 ± 0.6 bBC	1.8 ± 0.3 cdC
EK	6.0 ± 0.5 cdA	3.4 ± 0.5 bB	3.1 ± 0.2 cdB	2.2 ± 0.2 bBC	1.5 ± 0.1 dD
NS	4.9 ± 0.6 dA	3.4 ± 0.2 bB	3.6 ± 0.4 bcB	2.6 ± 0.4 bC	2.4 ± 0.4 abC
AM	9.0 ± 0.6 bA	5.0 ± 0.3 aB	4.9 ± 0.2 aB	2.9 ± 0.4 bC	2.1 ± 0.3 bcD
CP	5.6 ± 0.8 cdA	3.7 ± 0.6 bB	2.7 ± 0.2 dC	1.9 ± 0.4 bCD	1.6 ± 0.2 cdD
SP	10.4 ± 1.1 aA	6.1 ± 2.1aB	4.4 ± 0.8 abBC	4.1 ± 1.4 aBC	2.7 ± 0.6 aC

The concentration of P in the roots was also influenced by species and supplied P forms (Table 3.7). The root P concentration of all species was lower than the shoot P concentration in the respective plant species supplied with all forms of P and vice versa for those not supplied with P. This is an indication that, with absence of P, plants highly invest in the roots rather than in the shoot in order to 'look' for extra P in the soils. Root P concentration in plants supplied with KH_2PO_4 was significantly higher than root P concentrations in plants treated with all other P forms apart from African nightshade. Root P concentrations of Exotic kale, Ethiopian kale, cowpea and spider plant were the same, when supplied with other P forms apart from KH_2PO_4. With the absence of P, root P accumulation in the species was significantly lower than in species supplied with KH_2PO_4. Spider plant and cowpea did not show significant differences in root P concentration, when supplied with phytate, rock P, FePO4 and without P.

Table 3.7: Effect of P-treatment on root P concentration (g kg^{-1} dry mass) in different species of AIVs (mean ± standard deviation). Mean values marked with same small letters within a column indicate no significant differences among the species, mean values marked with same capital letters within a row indicate no significant differences among different P-treatments ($p > 0.05$, ANOVA, LSD test, n = 4); XK: exotic kale, EK: Ethiopian kale, NS: African nightshade, AM: amaranth, CP: cowpea, SP: spider plant.

Species	P-treatment				
	KH_2PO_4	Phytate	Rock P	$FePO_4$	Without P
	Phosphorus concentrations in the root (g kg^{-1}dry mass)				
XK	4.2 ± 0.7 bA	3.1 ± 0.2 bB	3.6 ± 0.4 aAB	3.2 ± 0.8 abB	2.2 ± 0.1 aC
EK	4.1 ± 0.5 bA	3.0 ± 0.4 bAB	2.8 ± 0.6 abB	2.8 ± 0.5 abB	1.8 ± 0.1 aC
NS	2.7 ± 0.4 cAB	3.1 ± 0.2 bA	2.2 ± 0.6 bB	2.7 ± 0.3 bAB	2.2 ± 0.1 aB
AM	6.1 ± 1.6 aA	4.5 ± 1.0 aB	3.0 ± 0.6 aC	3.4 ± 0.3aBC	2.2 ± 0.2 aC
CP	3.2 ± 0.5 bcA	2.2 ± 0.3 cB	2.0 ± 0.2 bB	1.6 ± 0.2 cB	2.3 ± 1.1 aB
SP	4.2 ± 0.7 bA	3.2 ± 0.3 bB	3.2 ± 0.5 aB	2.7 ± 0.4 abB	2.7 ± 0.1 aB

3.4.3.1 Phosphorus accumulation in vegetable species

When vegetable species were supplied with different P forms, the P accumulation in their tissues varied significantly among the treatments (Table 3.8). P accumulation was highest in all the species grown in soils supplied with KH_2PO_4 and lowest in soils, which were not fertilized with P. All species showed similar P accumulation, when supplied with KH_2PO_4 ranging from 17.8 mg $plant^{-1}$ for spider plant to 21.6 mg $plant^{-1}$ for cowpea.

Despite observing no significant changes in P accumulation among all vegetables species under KH_2PO_4 treatment, they ended up having different shoot fresh mass and total dry mass (Table 3.3 and 3.5). All the species supplied with phytate were significantly lower in P accumulation, when compared with KH_2PO_4 treatment, but were significantly higher than all species in rock P treatment. Nevertheless, P accumulation in exotic kale supplemented with Rock P was not significantly different from that supplied with phytate

Table 3.8: Effect of P-treatment on P accumulation (mg plant^{-1}) in different AIV species (mean ± standard deviation). Mean values marked with same small letters within a column indicate no significant differences among the species, mean values marked with same capital letters within a row indicate no significant differences among different P-treatments ($p > 0.05$, ANOVA, LSD test, n = 4); XK: exotic kale, EK: Ethiopian kale, NS: African nightshade, AM: amaranth, CP: cowpea, SP: spider plant.

Species	P-treatment				
	KH_2PO_4	Phytate	Rock P	$FePO_4$	Without P
	Phosphorus accumulation (mg plant^{-1})				
XK	18.9 ± 3.2 aA	10.1 ± 1.4 cB	7.1 ± 3.2 aBC	5.2 ± 0.2 aCD	1.9 ± 0.3 aD
EK	19.9 ± 1.4 abA	10.9 ± 0.7 cB	9.7 ± 1.4 aB	4.9 ± 0.3 aC	1.7 ± 0.1 abD
NS	19.1 ± 0.5 abA	14.0 ± 1.4 aB	3.5 ± 2.6 bC	1.5 ± 0.4 cCD	0.4 ± 0.1 cD
AM	19.2 ± 5.0 abA	13.7 ± 1.8 abB	8.5 ± 1.2 aC	3.5 ± 1.1 bD	0.9 ± 0.5 bcD
CP	21.6 ± 3.2 aA	14.0 ± 1.0 abB	8.8 ± 2.1 aC	3.2 ± 0.6 bD	1.9 ± 0.6 aD
SP	17.8 ± 1.0 aA	10.9 ± 1.1 bcB	1.5 ± 0.4 bC	1.4 ± 0.1 cC	0.7 ± 0.6 cC

Exotic kale, amaranth, cowpea and spider plant differed in the amount of P accumulated in phytate and KH_2PO_4 treatment, but ended up producing similar shoot fresh mass and total dry mass (Table 3.3 and 3.5). This signified, that the amount of P taken up by plants supplied with KH_2PO_4 was somehow in excess, leading to luxury uptake that was not useful to plants. When P was supplied as phytate, exotic kale, Ethiopian kale and spider plant had lower P accumulation than other species. African nightshade had the highest accumulation of P in phytate treatment, but the P accumulation was not significantly different from those in amaranth and cowpea. When rock P was supplied as P source, exotic kale, Ethiopian kale, amaranth and cowpea accumulated similar and significantly higher P amounts, whereas African nightshade and spider plant had the least accumulation. Ethiopian kale, amaranth and cowpea accumulated about two times higher P levels, when rock P was supplied, than when $FePO_4$ was used. Thus, Ethiopian kale, amaranth and cowpea had greater preference for rock P over $FePO_4$. It implies that rhizosphere processes like root-triggered processes (uptake, growth and release of solutes/acids), differed between species or within the species depending on the P treatment.

The acquisition of P from sparingly soluble treatments can be linked to either morphological root mechanisms of the plant by enhancing root growth rate, root hair formation (density and length), increased specific root length and reduced root diameter or modification of rhizosphere pH or release of phenolic compounds, phosphatase, carboxylates and mucilage to mobilize P (Lambers et al. 2006; Pearse 2011; Shen et al. 2011). Plant P accumulation in species not supplied with P was significantly lower than those supplied with KH_2PO_4, phytate and rock P in all species apart from spider plant. There were no significant differences in P accumulation, between species not supplied with P and those supplied with $FePO_4$ apart from Ethiopian kale.

The trend in P accumulation among the treatments was in agreement with Pearse et al. (2007), who used similar P treatments on *Lupinus albus*, *Lupinus cosentinii, Lupinus angustifolius*, *Brassica napus*, *Triticum aestivum*, *Pisum sativum* and *Cicer arietinum*. Vegetable species are known to accumulate different amounts of nutrients, including P, in their organs (Schachtman et al. 1998; Ramaekers et al 2010). Effective transport of P within the plant, across plasma membrane, is facilitated by high Pi concentration in the cytoplasm, P-transporters and negative membrane potential (Schachtman et al. 1998). At the same time, species had different ability to take up and translocate P at different plant stages and thus each species had to build up different amounts of biomass depending on developmental stage of vegetable species (IPNI 1999; Veneklaas et al. 2012). Li et al. (2011), review article stated that critical plant yield depends on species characteristics. The amount of P in species under highly soluble P implied, that the amount of P taken up by the plants does not only determine the dry mass of the plant, but is effective in other processes within the plant. Compared to other vegetable species, African nightshade effectively acquired more P in organic and highly soluble P treatments, but not in other forms of P. African nightshade can be a good indicator for presence or absence of P in the soil since it does well in places with readily available P. Cowpea had an overall ability to acquire P in almost all the treatments in comparison to all other species and that's why it had the highest yield and total biomass (Table 3.3 and 3.4). Cowpea is a high P use efficient species because of its ability to produce high biomass in low P soils. This is in agreement with the recommendations stated by Ansah et al. (2016).

3.4.3.2 Biomass production per unit of plant accumulated P

The measurement of biomass produced per unit P is termed internal phosphorus use efficiency (IPUE). IPUE was measured to identify vegetable species that can achieve more yields per unit P uptake from the soil. The IPUE is affected by the presence or absence of P fertilizer in the soil. The study used different P treatments, which lead to different levels of P accumulation in plants (Table 3.8).

The IPUE of plants supplied with KH_2PO_4 was significantly lower than that supplied with phytate in all the species apart from cowpea and spider plant (Table 3.9). The IPUEs for cowpea and spider plant were similar, when supplied with KH_2PO_4 or phytate. African nightshade and cowpea obtained the highest IPUE among the species, while amaranth and spider plant had the least IPUE, when supplied with KH_2PO_4.

Table 3.9: Effect of species and P treatment on internal P utilization efficiency (g mg^{-1} P accumulation) in different AIV species (mean ± standard deviation). Mean values marked with same small letters within a column indicate no significant differences among the species, mean values marked with same capital letters within a row indicate no significant differences among different P-treatments ($p > 0.05$, ANOVA, LSD test, n = 4); XK: exotic kale, EK: Ethiopian kale, NS: African nightshade, AM: amaranth, CP: cowpea, SP: spider plant.

Species	P-treatment				
	KH_2PO_4	Phytate	Rock P	$FePO_4$	Without P
	Internal P utilization efficiency (g mg^{-1} P)				
XK	0.16 ± 0.03 bD	0.31 ± 0.03 aBC	0.27 ± 0.06 bcC	0.36 ± 0.05 bcB	0.52 ± 0.06 abcA
EK	0.18 ± 0.01 bD	0.30 ± 0.04 aC	0.33 ± 0.01 bC	0.42 ± 0.02 bB	0.62 ± 0.02 aA
NS	0.23 ± 0.02 aC	0.30 ± 0.01 aB	0.31 ± 0.04 bB	0.39 ± 0.04 bA	0.44 ± 0.08 bcA
AM	0.12 ± 0.01 cD	0.21 ± 0.02 bC	0.22 ± 0.01 cC	0.34 ± 0.04 bcB	0.46 ± 0.04 bcA
CP	0.20 ± 0.04 abC	0.30 ± 0.04 aBC	0.40 ± 0.04 aB	0.55 ± 0.11 aA	0.57 ± 0.11 abA
SP	0.10 ± 0.01 cC	0.19 ± 0.06 bBC	0.25 ± 0.04 cB	0.27 ± 0.08 cB	0.41 ± 0.11 cA

IPUE did not significantly differ between the phytate and Rock P treatment in all species. Between species, Ethiopian kale, nightshade and cowpea had the highest IPUE, when supplied with phytate compared with other species. Comparing poorly soluble P (rock P and $FePO_4$), the IPUE of all vegetables supplied with FePO4 were significantly higher than that supplied with rock P apart from spider plant. Cowpea had the highest IPUE in both treatments, rock P and $FePO_4$. The IPUE of plants not supplied with P was significantly higher than those supplied with KH_2PO_4, phytate or rock P, where for instance, Ethiopian kale, amaranth and spider plant showed a more than 3-fold higher IPUE when soils were not supplied with P compared to that, when supplied with KH_2PO_4. Species not supplied with P had also significantly higher IPUE than those supplied with $FePO_4$ apart from African nightshade and cowpea. The plants not supplied with P had a high IPUE to enable them to produce biomass with limited available P derived from the seeds, thus maximizing biomass production from all P available. Ethiopian kale and cowpea had a higher IUPE when grown without P, whereas spider plant had the least IPUE, compared to other species.

Many studies have looked at genotypic variation of IPUE among species (Greenwood et al. 2005; Hammond et al. 2009; Rose et al. 2011, 2016; Ansah et al 2016) and the IPUE among species in P starved soils (Simpson et al 2011; Plaxton and Tran 2011; Pant et al. 2015), but not along the P forms. Cowpea had the highest IPUE under all P forms indicating that it can yield more than other plant species when supplied with same P amount or in limited amount, because it has a lower requirement for P in biomass production. The identified species (Ethiopian kale and cowpea, to some extend exotic kale) have the capacity to grow well in P limited soils or with minimal application of P fertilizer, which could reduce the need for P fertilizer. Crops that are more efficient in P uptake and utilization, produce higher yields since they have low internal P demand for growth and metabolic activities (Balemi and Negisho, 2012).

3.4.4 Morphological root traits

Plant allocation traits considered in our study were; root to shoot ratio, specific root length, root diameter and root hair density. Root morphological traits were measured in H_2PO_4, rock P and P deficient treatments. Phytate and $FePO_4$ treatments were not considered for determination of morphological root traits, because phytate supply resulted in similar results in biomass production as KH_2PO_4 supply did and $FePO_4$ and rock P treatments resulted in similar biomass production as well, which was seen in the previous paragraphs.

3.4.4.1 Root length to shoot biomass ratio

Comparing the root length of vegetable species with corresponding biomass was fundamental in order to determine their relationships and how this relationships were affected by P forms and P rates. The results showed that, exotic kale, Ethiopian kale and amaranth did not show any differences in their ratios under KH_2PO_4, rock P and without P treatments. (Table 3.10). On the other hand, African nightshade, cowpea and spider plant had a lower root length to shoot biomass in plants supplied with KH_2PO_4, than those not supplied with P. Therefore, growth of biomass of the kales and amaranth was not related to root length, while within the remaining species, root length was positively related to plant biomass. This study agrees with Leon and Schwang (1992), who found the yield of barley and oat cultivars to be related to root length. The study indicated that plants did not invest much in root length, when supplied with highly soluble P (and to some extent rock P) and rather invested in shoot biomass, unlike in soils not supplied with P.

Table 3.10: Effect of P-treatment on root length to shoot biomass ratio (m g^{-1}) of different vegetable species (mean ± standard deviation). Mean values marked with same small letters within a column indicate no significant differences among the species, mean values marked with same capital letters within a row indicate no significant differences among different P-treatments ($p > 0.05$, ANOVA, LSD test, n = 4).

Species	P-treatment		
	KH_2PO_4	Rock P	Without P
	Root length to shoot biomass ratio (m g^{-1})		
Exotic kale	55 ± 12 aA	62 ± 13 abA	91 ± 45 bA
Ethiopian kale	46 ± 6 abA	38 ± 5 bcA	78 ± 36 bcA
African nightshade	40 ± 8 bB	83 ± 34 aB	163 ± 33 aA
Amaranth	36 ± 3 bA	43 ± 3 bcA	45 ± 9 cA
Cowpea	21 ± 3 cB	26 ± 6 cB	39 ± 3 cA
Spider plant	46 ± 8 abB	79 ± 11 aAB	90 ± 31 bA

Vegetable species differed greatly in the root length to shoot biomass ratio. When KH_2PO_4 was used as a source of P, exotic kale showed the highest ratio, while cowpeas had the least. On the other hand, African nightshade had the highest root length to shoot biomass ratio in P depletion treatment, whereas cowpea and amaranth had the least. Considering KH_2PO_4 treatment, cowpea accumulated significantly higher shoot biomass compared to exotic kale (Table 3.3). In KH_2PO_4 treatment, higher root length to shoot biomass ratios in African nightshade and spider plant lead to low P accumulation and thus significantly lower shoot biomass compared to other vegetables species. The same behavior among species was manifested in soils without P, where African nightshade had the highest ratio among the species. Thus, African nightshade, cowpea and spider plant responded to increasing P demands, with an increase in root length, which results in higher ratios. Whereas, in exotic kale, Ethiopian Kale and amaranth ratios did not significantly alter between P forms.

3.4.4.2 Specific root length of AIVs

Specific root length was calculated by measuring the vegetables root length divided by root biomass. Specific root length varied among species and among P treatments of individual species (Table 3.11). Exotic kale, Ethiopian kale and spider plant did not increase or decrease their specific root length in all the P treatments since their differences were not significant. African nightshade and cowpea significantly increased their specific root length when soils were not supplied with P and in rock P treatment compared to KH_2PO_4 treatment. However, the specific root length of amaranth was significantly lower in soils unfertilized with P compared to KH_2PO_4 treatment, but higher in soils supplied with rock P.

Past studies associated P deficiency with enhanced root length or specific root length in some crop species (Vance et al. (2003), wheat (Manske 2000; Liao et al. 2008), *Brassica oleracea* (Hammond et al 2009), beans (Miller et al. 2003), *Brassica napus* (Lyu et al. 2016) and Rytidosperma species (Waddell 2017). Our results showed, that most of the vegetable species did not modify their root length in order to acquire more P, neither in soils containing plant available P nor in soils without P/adsorbed P. Increased root length was only observed in cowpea and African nightshade, which increased their root length in P deficient soil and in soil containing sparingly soluble P compared to soil containing plant available P.

Table 3.11: Effect of P-treatment on specific root length (m g^{-1}) of different AIV species (mean ± standard deviation). Mean values marked with same small letters within a column indicate no significant differences among the species, mean values marked with same capital letters within a row indicate no significant differences among different P-treatments ($p > 0.05$, ANOVA, LSD test, n = 4).

Species	P-treatment		
	KH_2PO_4	Rock P	Without P
	Specific Root length (m g^{-1})		
Exotic kale	231 ± 15 bA	208 ± 17 abcA	270 ± 91 aA
Ethiopian kale	188 ± 21 cA	168 ± 27 bcA	215 ± 77 abcA
African nightshade	136 ± 24 dB	270 ± 114 aA	290 ± 66 aA
Amaranth	192 ± 21 cB	230 ± 16 abA	154 ± 28 bcC
Cowpea	100 ± 7 eB	141 ± 10 cA	127 ± 19 cA
Spider plant	316 ± 37 aA	271 ± 12 aA	229 ± 80 abA

Our study showed, that increase in specific root length is a carbon efficient mechanism by the roots for increasing rhizosphere soil exploration (Waddell et al. 2017), which leads to increased rates of P uptake (Brown et al. 2013).

Among all the vegetable species, cowpea had the least specific root length, when P was supplied in all the three forms. When P was supplied as KH_2PO_4, spider plant had the highest specific root length. The specific root length decreased in the order; spider plant > exotic kale > Ethiopian kale and amaranth > African nightshade > cowpea. On the other hand, when plants were supplied with rock P, African nightshade and spider plant acquired the highest specific root length although not significantly different from the specific root length obtained by exotic kale and amaranth. However, exotic kale and African nightshade had higher specific root lengths in soils not fertilized with P, although not significantly different from Ethiopian kale and spider plant. Cowpea showed least responses to reduced P availability with lower specific root length. The ability to increase root length in P deficient soils led to higher yield and total dry mass found in the kales compared to other species. In rock P, the increased specific root length of African nightshade and spider plant did not contribute to biomass production.

3.4.4.3 Root diameter for AIVs

There were significant differences in root diameter among species ($p < 0.05$) as well as between species and phosphorus forms ($p < 0.05$) interactions, while no significant differences were observed among P forms ($p = 0.23$; Table 3.12). There were no differences in root diameter among the P treatments of exotic kale, Ethiopian kale and cowpea. The root diameter of spider plant species supplied with KH_2PO_4 was significantly lower than that supplied with rock P and without P.

Table 3.12: Effect of P-treatment on root diameter (mm) of different AIV species (mean ± standard deviation). Mean values marked with same small letters within a column indicate no significant differences among the species, mean values marked with same capital letters within a row indicate no significant differences among different P-treatments ($p > 0.05$, ANOVA, LSD test, n = 4).

Species	P-treatment		
	KH_2PO_4	Rock P	Without P
	Root diameter (mm)		
Exotic kale	0.23 ± 0.01 deA	0.27 ± 0.02 cdA	0.27 ± 0.06 cdA
Ethiopian kale	0.27 ± 0.02 cA	0.31 ± 0.03 bA	0.32 ± 0.05 bA
African nightshade	0.31 ± 0.03 bA	0.30 ± 0.02 bcA	0.23 ± 0.02 dB
Amaranth	0.25 ± 0.01 cdB	0.23 ± 0.01 eB	0.30 ± 0.03 bcA
Cowpea	0.43 ± 0.03 aA	0.40 ± 0.02 aA	0.42 ± 0.01 aA
Spider plant	0.21 ± 0.02 eB	0.26 ± 0.01 deA	0.26 ± 0.03 cdA

African nightshade had a smaller diameter of roots in treatments without P compared to supply with KH_2PO_4 or Rock P. A smaller root diameter has the ability to explore a bigger soil volume per unit root surface area. Although thinner roots can be efficient in acquiring P, the carbon cost of maintaining and producing finer roots may be high due to frequent replacement (Gahoonia1 and Nielsen 1996). Amaranth obtained a thicker root diameter in soils without P. It was able to maintain intermediate P accumulation and total dry mass. Spider plant showed a thicker diameter in sparingly soluble P and in P deficiency treatments compared to soluble P, which was not useful in P acquisition and biomass accumulation. When all the vegetables were grown under same P treatment, the result showed that growing them in KH_2PO_4 resulted in highest root diameter in cowpea (0.43 mm) and lowest root diameter in spider plant (0.21 mm), which was not significantly different from exotic kale (0.23 mm). Cowpea grown in rock P and without P treatment had the highest diameter of all species in both treatments. Amaranth supplied with rock P showed smallest root diameter among species. When P was not supplied to the soils, African nightshade developed thinnest roots. Exotic kale was among the species that showed thinnest roots. With this reduced root diameter, exotic kale was able to acquire more P (table 3.8) and biomass (Table 3.5), than other species.

3.4.4.4 Root hair density

Vegetable species differed in root hair density under different P treatments (Table 3.13). The root hairs were counted from a set of 10 roots derived from each species and from each treatment and then the average was used. The root hair appearance is displayed in figure 3.3. There was no significant difference in the specific root hair density of exotic kale, Ethiopian kale and African nightshade, when supplied with the three P forms. Instead, amaranth and spider plant had significantly lower root hair density when supplied with KH_2PO_4 and rock P than when not supplied with P. Cowpea showed a significantly decreased root hair density in P deficient soil compared to soil supplied with KH_2PO_4.

Previous studies have found out that deficiency of nutrients, especially P, stimulates root hair production leading to higher root hair density and length (Lambers et al. 2006; Hammond et al. 2009; Peret et al. 2011). In our study, the findings of spider plant and amaranth agreed with the past study in other crop species (Williamson et al. 2001; Hammond et al 2009; Niu et al. 2013; Vejchasarn et al 2016). Production of more root hairs among vegetables in soils deficient in P may be due to root cortical cells providing more sites where trichoblasts can grow, thus allowing production of numerous root hairs (Ma et al. 2001) or stimulation and presence of auxin and ethylene (Ma et al. 2001; Neumann and Martinoia 2002). The high stimulation of root hair density may lead to short main root as seen in *Arabidopsis* grown in P deficient soils (Williamson 2001). This is also displayed in the vegetable species in figure 3.4. There were no differences among species in root hair density when P was supplied in the form of KH_2PO_4. When P was supplied as rock P, exotic kale obtained a significantly higher root hair density than African nightshade, but there were no differences in the other species. Also, Ethiopian kale, amaranth, cowpea and spider plant had almost the same root hair density that was not significantly different from African nightshade when grown in soils supplied with rock P. In P deficient soils, spider plant had highest and cowpea lowest root hair density, while the remaining vegetable species had an almost similar density.

The root hair density among species did not play a vital role in biomass production. For instance, the kales and cowpea had a higher shoot mass and total dry weight in soils unfertilized with P, but they acquired an intermediate and lower root hair density.

Table 3.13: Effect of P-treatment on root hair density (number per mm of root) of different AIV species (mean ± standard deviation). Mean values marked with same small letters within a column indicate no significant differences among the species, mean values marked with same capital letters within a row indicate no significant differences among different P-treatments ($p > 0.05$, ANOVA, LSD test, $n = 4$).

Species	P-treatment		
	KH_2PO_4	Rock P	Without P
	Root hair density (number of hairs per mm root)		
Exotic kale	9.5 ± 4.1 aA	11.2 ± 3.9 aA	10.8 ± 3.4 bA
Ethiopian kale	10.1 ± 5.0 aA	9.1 ± 2.8 abA	11.1 ± 4.2 bA
African nightshade	6.4 ± 2.4 aA	5.0 ± 2,6 bA	5.7 ± 2.0 bcA
Amaranth	5.1 ± 2.9 aB	6.2 ± 1.8 abAB	9.9 ± 3.2 bA
Cowpea	7.7 ± 2.3 aA	6.9 ± 2.2 abAB	4.1 ± 1.6 cB
Spider plant	7.3 ± 2.7 aB	9.7 ± 6.8 abB	19.0 ± 5.4 aA

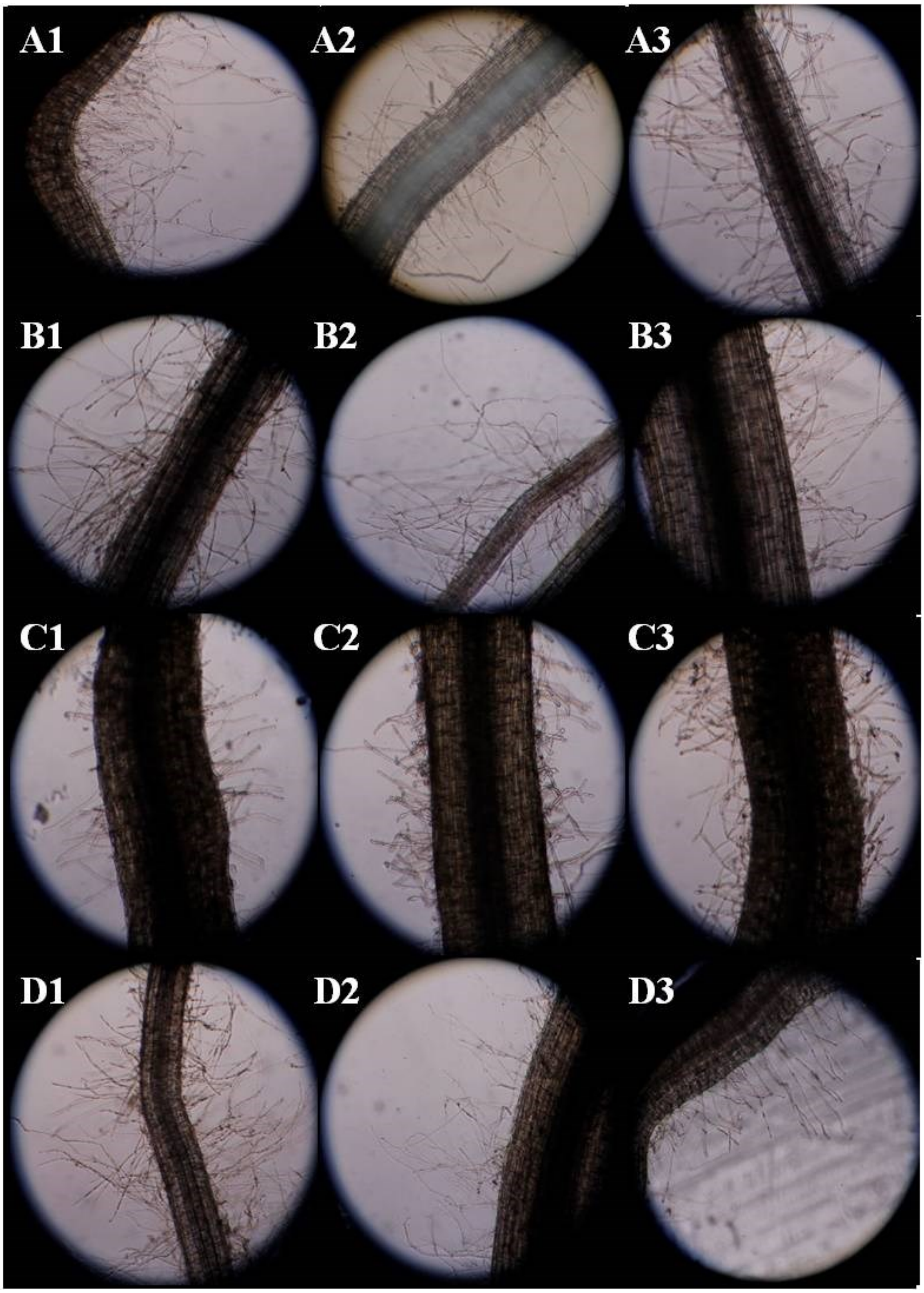

Figure 3.3: Visual representation of root hairs as seen under a microscope with lens diameter of 1 and 2 mm; where A – exotic kale, B – amaranth, C – cowpea, D – spider plant; 1 – without P, 2 – Rock P and 3 – KH_2PO_4 treatment.

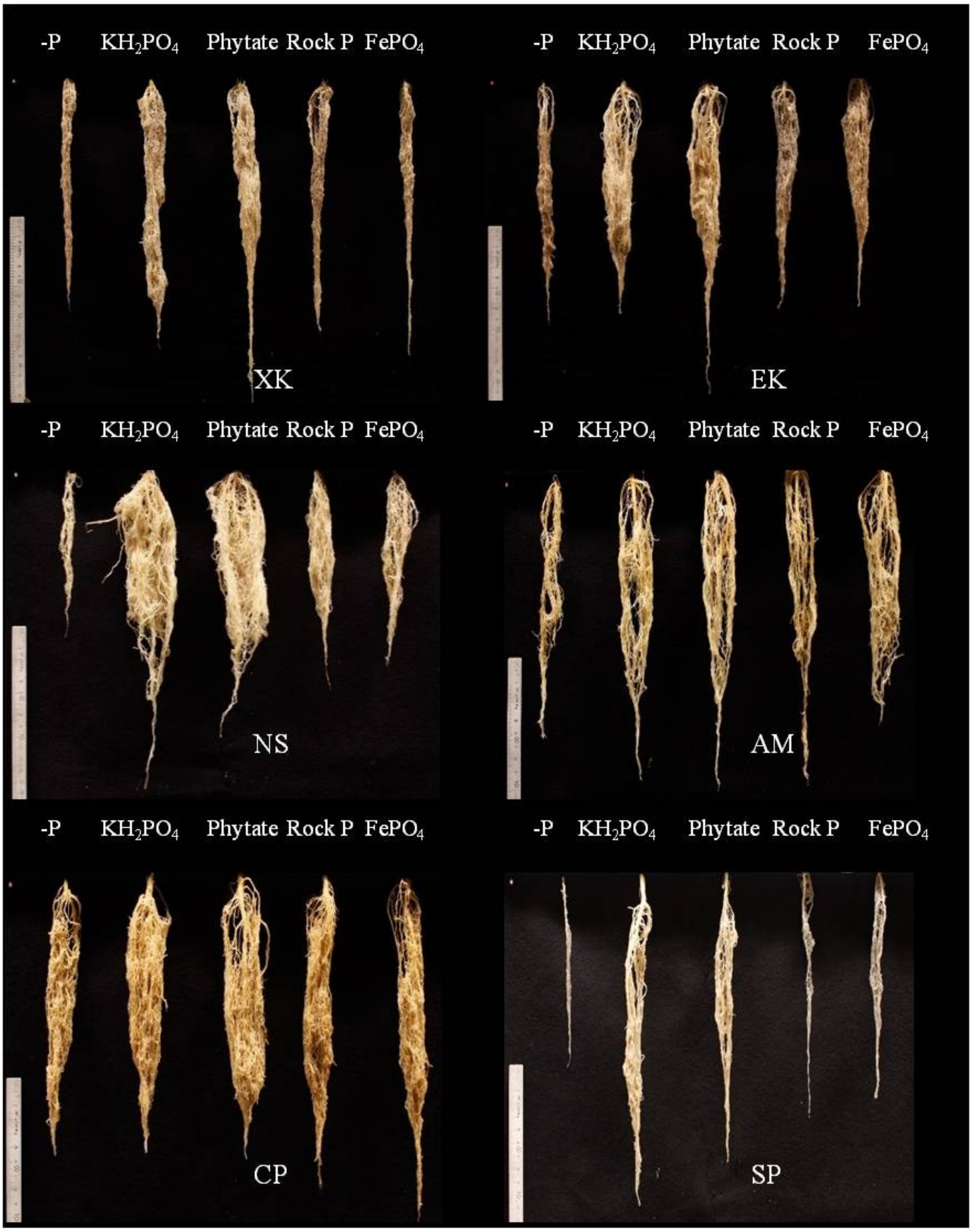

Figure 3.4: Effect of P treatments on the root system of various vegetable species (visual representation of each species in different P treatments); where XK – exotic kale, EK – Ethiopian kale, NS – African nightshade, AM – amaranth, CP – cowpea and SP – spider plant.

3.4.5 Rhizosphere pH levels

Rhizosphere pH levels influenced by vegetable species were measured to determine species that can lower or increase the pH of plant surrounding soils for effective uptake of P from the soil. The increase or decrease of pH may be facilitated by exuding of compounds from the roots.

The results showed that exotic kale, Ethiopian kale and spider plant responded by increasing the pH when supplied with P treatments unlike other species that reacted by lowering the pH in all the P treatments (Table 3.14). The rhizosphere pH of exotic kale was higher and almost similar when P was supplied as KH_2PO_4, phytate and rock P, while in $FePO_4$ and soil unfertilized with P it had a lower pH (less than 6). However, the pH under $FePO_4$ for exotic kale was not significant different from rock P, phytate or KH_2PO_4. Nevertheless, Ethiopian kale showed the highest rhizosphere pH in phytate and rock P treatment, whereas there were no significant differences between pH under KH_2PO_4 and $FePO_4$ supply.

Table 3.14: Effect of P-treatment on soil leachate pH of different AIV species (mean ± standard deviation). Mean values marked with same small letters within a column indicate no significant differences among the species, mean values marked with same capital letters within a row indicate no significant differences among different P-treatments ($p > 0.05$, ANOVA, LSD test, n = 4); XK: exotic kale, EK: Ethiopian kale, NS: African nightshade, AM: amaranth, CP: cowpea, SP: spider plant.

Species	P-treatment				
	KH_2PO_4	Phytate	Rock P	$FePO_4$	Without P
			Leachate pH		
XK	6.2 ± 0.7 aA	6.3 ± 0.9 aA	6.0 ± 1.3 abA	5.1 ± 0.7 abAB	4.3 ± 0.1 bcB
EK	6.4 ± 0,4 aAB	6.8 ± 0.4 aA	7.0 ± 0.4 aA	5.7 ± 0.8 aB	4.5 ± 0.2 bC
NS	3.8 ± 0.4 bB	4.7 ± 0.4 bA	4.6 ± 0.4 cA	4.6 ± 0.2 bcA	4.6 ± 0.2 bA
AM	3.6 ± 0.3 bC	3.4 ± 0.1 cC	4.6 ± 0.2 cA	4.0 ± 0.1 cB	4.2 ± 0.2 cdB
CP	4.0 ± 0.4 bB	4.2 ± 0.3 bB	5.4 ± 0.9 bcA	4.4 ± 0.5 bcB	4.0 ± 0.2 dB
SP	6.7 ± 0.5 aA	6.8 ± 0.4 aA	4.9 ± 0.2 bcB	4.9 ± 0.1 bB	4.9 ± 0.2 aB

Ethiopian kale that grew in unfertilized P soils lowered the pH to 4.5, which was significantly lower than in any other P treatment. The pH levels of soils with African nightshade were reduced below 6.0 in all the P forms to a similar rhizosphere pH of 4.6 – 4.7, apart from the rhizosphere pH of 3.8 in KH_2PO_4 treatment, which was significantly lower. Like African nightshade, amaranth reduced rhizosphere pH to lower values than 6.0. Amaranth showed a higher pH in rock P treatment followed by $FePO_4$ and P unfertilized soils, which had almost the same pH. Amaranth in phytate and KH_2PO_4 treatment recorded the lowest pH compared to other P treatments. Cowpea lowered rhizosphere pH to a similar extend like shown in nightshade and amaranth for all P treatments, except in the rock P treatment, where the highest pH (pH 5.4) was measured. All other P treatments of cowpea lead to similar rhizosphere pH. Spider plant grown in phytate and KH_2PO_4 treatments had significant higher pH values of 6.8 and 6.7, respectively, than those from the remaining P treatments.

Previous studies have also shown that a number of species, such as soybean (Shujie and Yunfa 2011), *L. albus (*Pearse et al. 2006), wheat and barley (Gahoonia and Nielsen 1996) decrease the rhizosphere pH in order to cope with P deficiency. Acidification of soil rhizosphere is associated with dissociation of organic acid to discharge anions (Hinsinger 2001).

The soil pH of exotic kale increased with increase in P accumulation in vegetable species. This was in agreement with the previous study by Pearse et al. (2006) on *Triticum aestivum* and *Lupinus albus*. The form in which P exists in soil solution was mostly affected by the rhizosphere pH. Uptake levels of P are highest between pH 5.0 - 6.0, where monovalent $H_2PO_4^-$ dominates (Schachtman et al. 1998). Plants can either increase or reduce the optimal soil pH for effective uptake of P. Except for pH above 8, rock P had increasing solubility with decreasing pH, whereas $FePO_4$ had an increasing solubility with increasing pH (Hinsinger 2001). That is why African nightshade, amaranth and cowpea obtained higher P in rock than $FePO_4$. It can be attributed that vegetables with the capacity to increase soil pH are able to perform better in $FePO_4$, but for our case, the vegetables species lowered the pH, thus performed poorly compared to the same species under rock P. They ended up reducing the pH in both P treatments favoring the uptake of rock P. The ability to produce similar yield (shoot fresh mass) and dry mass for exotic kale (Table 3.3 and 3.4) grown in rock P and $FePO_4$ can be attributed to their rhizosphere pH. This was because both treatments had their pH within optimal level when exotic kale was grown.

In phytate and KH_2PO_4, the kales and spider plant increased their pH beyond 6.0, while the remaining vegetable species reduced their soil pH. Hinsinger et al. (2003), link the increase in soil pH to increased NO_3^- uptake by vegetable species, causing higher release of OH^- or HCO_3^- or increased uptake of H^+ to balance the soil ion charges. The reason why the kales performed well in $FePO_4$, was because, unlike other vegetables species, their pH was within the optimal range necessary for plants to acquire P. Other species in $FePO_4$ treatments lowered rhizosphere pH from the initial soil pH, which reduces the ability to extract P from the soil and that's why most vegetable species with pH below the normal 5.0 – 6.0 in $FePO_4$ treatment extracted less P from the soil, resulting in lower P accumulation per plant (Table 3.8.) Previous study by Rose et al. (2010) showed wheat to increase the rhizosphere pH in order to acquire the adsorbed P in the soils, which was in agreement with Ethiopian kale in our study. Ethiopian kale had a higher pH of above 6, but was able to take up more P from rock P treated soils resulting to higher biomass compared to other species. Thus, the distribution of P in most species is determined by solution pH, which leads to dissociation of orthophosphoric acid to produce phosphate ions (Hinsinger 2001).

In treatments without P, all the plants had to lower their pH below 5.0 in order to acquire the little P available in the soil. Cowpea had the lowest pH and that's likely the reason it had higher P concentration and biomass.

3.5 General discussion and recommendation

In agricultural ecosystems, P is increasingly lost from soil through reactions with numerous soil constituents, greatly reducing bioavailability of soil P to plants. Our results indicated that vegetables behaved and responded differently when supplied with different P forms. From the experiment, deficiency of P did not become visible (Fig. 3.1) by change of leaf colour i.e. reddish- purple colour caused by sugar accumulation resulting in anthocyanin pigments (Gruhn et al. 2000), but most species showed deficit through retardant growth. Hence with increasing availability /solubility of the P source more biomass was produced in the vegetable species studied. Our vegetables under study, had more biomass and P accumulation in KH_2PO_4 and phytate treatments, because these P forms are highly soluble and therefore easily available to plants. It is well known, that diffusion of Pi to the roots can be improved by increasing its concentration in soil solution (Balemi and Negisho, 2012). Although all species responded well to KH_2PO_4, African nightshade and cowpea produced the highest total dry mass (shoot and root), while cowpea alone produced the highest yield (shoot fresh mass). However, $FePO_4$ was most efficiently utilized by Ethiopian kale and exotic kale while rock P was efficiently utilized by cowpea and Ethiopian kale. The efficiency for utilization of sparingly soluble P forms (either $FePO_4$ or rock phosphate) was low in African nightshade and spider plant, thus they are not suitable for growth in P absorbing soils. Rock P and $FePO_4$ are known for their slow P release into the soil solution, thus low availability to the plant. Plant species that grow well in sparingly soluble P are most suited in acidic and/ or basic soils, where P is limited by adsorption. The ability for cowpea to produce high yields in all the P forms in relation to the plant's P accumulation shows that it has the highest IPUE.

Cowpea had the ability to build more biomass under low P conditions and produced more than other species given the same amount and forms of P, thus can be a cost effective species in terms of P fertilizer application. Cowpea is a leguminous plant, which also used N_2 from the atmosphere and fixed it to the plants through the root. With its higher IPUE and its ability to fix N_2, the use of cowpea can reduce the cost of purchasing P and N fertilizers.

Vegetable species adopted different strategies in order to cope with P availability. Vegetables grown in soil containing highly soluble P, invested more in the shoot biomass and less in the root biomass, while those grown in P deficient soils invested more biomass in the roots than in shoots. It is known that P deficiency (as well as N and S deficiency) favors the allocation of biomass to the roots (Péret et al. 2011). Thus, allocation of more carbon to roots, leads to higher root exploration in the soil and increased root growth (Balemi and Negisho, 2012), to access extra P to produce a reasonably high yield (Balemi and Negisho, 2012). Despite vegetables developing large root to shoot ratios in P deficient soils, other mechanisms (i.e. plant physiological and morphological traits) play a role in ensuring that the species grows well and yields. This explains why African nightshade had higher root to shoot ratio, but ended up accumulating less plant P and lowest dry matter.

On the other hand, African nightshade and cowpea reacted to P deficiency by increasing specific root length unlike other vegetable species, which showed almost the same root length in treatments without P and with soluble P (KH_2PO_4). This facilitated the production of plant biomass by decreasing carbon cost for the formation of roots. Cowpeas with elongated specific root length showed a high yield, which was attributed to its ability to form more biomass per unit P. Decrease of rhizosphere pH by Ethiopian kale, exotic kale and spider plant in order to efficiently acquire rock P makes them most suitable to be grown in calcareous soil. Thus, plants showed varied traits, which were independent of one another. Some of these identified responses are likely to be genetically influenced (Peret et al. 2011) or a result of complex interactions in the rhizosphere leading to availability or adsorption of P (Ansah et al. 2016), because they only occurred in specific vegetable species. These traits or strategies exist and work independently, however, when combined they may work effectively to acquire higher P levels leading to higher yield and total plant biomass.

Chapter 4: Quantification of nutrient fluxes in AIVs: Effects of species, harvesting techniques and production systems on nutrient export from soil

4.1 Abstract

Intensification of agriculture due to population increase has led to negative soil nutrient balances in sub Saharan Africa, hence low food production. In vegetable production systems, nutrients from the soil are exported to the markets via harvesting of the edible organs and at some point non-edible organs (residues) go along with edible ones. Variation in soil fertility associated with agronomic measures like harvesting methods and production systems have been overlooked. For that case, a field experiment was conducted to determine species and site-specific, harvest related nutrient exports by individual vegetable species in order to provide information on species-specific fertilizer needs for farmers. Six leafy vegetables (spider plant - *Cleome gynandry*, cowpea - *Vigna unguiculata*, amaranth - amaranthus *cruentus*, African nightshade - *Solanum scabrum*, Ethiopian kale - *Brassica carinata* and exotic kale - *Brassica oleracea*) were cultivated in batch and continuous production systems and harvested by picking, cutting and pulling. The results showed that spider plant had the ability to remove higher levels of N (6.2 - 9.9 kg N per 10^3 kg leaf fresh mass) and P (1.41 – 2.40 kg P per 10^3 kg leaf fresh mass). Amaranth exported high amount of K (17.2 – 18.9 kg K per 10^3 kg leaf fresh mass) and Mg (0.94 – 1.65 kg Mg per 10^3 kg leaf fresh mass), while Ethiopian kale, exotic kale and spider plant exported a lot of sulphur from the soil to the market. Pulling of vegetables during harvesting, removed a lot of nutrients compared to picking and cutting methods, although species differed in the amount removed under the production systems. The continuous production system removed a lot of nutrients from the soil, especially under cutting and pulling harvesting techniques. Therefore, better crop management practices should be used to decrease the export of nutrients and reduce the amount of fertilizer applied as soil nutrients amendments.

Key words: Vegetable production systems, crop management, soil fertility, nutrient export, fertilizer needs.

4.2 Introduction

4.2.1 Soil fertility status of small holder farming systems in sub-Saharan Africa

Soil fertility degradation is among the major factors that have contributed to low food production in sub-Saharan Africa (sSA) among small scale farming systems. For a longer period, land has been overworked and continuous growing of crops has led to removal of soil nutrients that were not replenished. Nutrient balances, both at national and regional levels of sSA, have shown relatively large negative variations, which are attributed to soil forming factors, resource management, wealth categories and distance from homesteads (Zingore et al. 2007). The soils are more acidic pH < 5.5 and low in soil organic matter (World Bank 1998). In addition sSA soils are dominated with sandy loam and clay loam categories and thus low in macro nutrients (N, P, K, S and Mg) and micro nutrients (Zn and B). Stoorvogel et al. (1993) projected that the average nutrient loss by 2000 for sub-Saharan Africa was 26 kg N, 3 kg P, and 19 kg K ha^{-1} $year^{-1}$. Narrowing it down, under same management and environmental conditions in Tanzania, maize and beans removed almost 58 kg N ha^{-1} $year^{-1}$, 13 kg P ha^{-1} $year^{-1}$and 56 kg K ha^{-1} $year^{-1}$ (Micheni et al. 2011), while in Ethiopia, nutrient depletion rate averages 122 kg N ha^{-1} yr^{-1}, 13 kg P ha^{-1} yr^{-1} and 82 kg K ha^{-1} yr^{-1} (Haileslassie et al. 2005). In Kenya maize alone is able to remove 25, 6 and 7 kg ha^{-1} $year^{-1}$ of N, P, K respectively from harvested products and 11, 2, 24 kg ha^{-1} $year^{-1}$ of N, P, K respectively from crop residues (FAO 2004b). Smaling et al. (1993), measured the aggregated nutrient balance in Kisii county, one of the major producers of vegetables, especially AIVs, in Kenya to be -112 kg N, -3 kg P and -70 kg K ha^{-1} yr^{-1}.

Poor soil fertility among the small holder farmers has been largely associated with inappropriate soil management techniques (crop removal, overgrazing, deforestation and converting marginal lands to agriculture use) and the nature of the soil, which accounts for large volume of nutrient losses in sSA (Omotayo and Chukwuka 2009). In most cases, high yield results in high nutrient export from the field to either market or store and vice versa. Harvested crop parts and removal of plant residues for fuel or feeds removes about 37% of the nutrients from the soil (Oenema et al. 2003). Drechsel, (2002), estimated crop harvests (products and residues) in addition to erosion, to contribute for about 70 % of all N losses, nearly 90% of all K losses, and 100% of the P losses in sub-Saharan Africa. Soil erosion highly depends on the slope. The water runoff deposits the nutrients in water bodies, causing eutrophication (Nyenje et al. 2010). Another source of soil nutrient loss is leaching, where N and P get leached from the soil depth at which plants can easily obtain these nutrients. Since soils in sub-Saharan Africa might be acidic in nature, denitrification is highly facilitated, where N in form of nitrate (NO_3) is converted to inert nitrogen gas (N_2) and lost to the atmosphere (Stoorvogel et al. 1993).

Use of mineral fertilizer has been seen as the most appropriate way of restoring soil fertility and crop yield. Appropriate use of mineral fertilizer has resulted in potential yield increase over years, although most small scale farmers are unable to use the right quantity at the right time and place due to high prices and its unavailability (Vanlauwe et al. 2014). Soil quality among small scale farms is spatially heterogeneous (Tittonell et al. 2008) and a number of crops are grown, which require different nutrient levels. That is why the existing blanket recommendation of mineral application cannot be an effective approach.

Farmers also replenished soil nutrients by addition of organic resources (majorly animal manure, composite and crop residues), which play an important short- and long-term role in nutrient availability and organic matter maintenance (Palm et al. 2001). The organic nutrient sources also minimize soil erosion and improve water retention capacity of the soil. Thus, it is the appropriate method for small scale farmers, who can use crop residues as mulch or incorporate them into the soil. But due to competing uses of harvested residues among small holder farmers, there might be not enough organic residues to restore soil fertility. Small scale, poor farmers use the residues as feed for animals, as fuel to supplement other forms of energy or burn them for other uses, which should be discouraged at farming level (Yevich and Logan 2003).

Other ways through which nutrients can be added to the soil, is by wet and dry deposition as a result of rain fall or deposition with dust. This method only applies to a few minerals and thus its contribution is less felt. However, N in the atmosphere can be fixed by biological N_2 fixation, which mostly happens by rhizobia living in symbiosis with leguminous plants (Stoorvogel et al. 1993). That adds almost 60% of N needed by the plant either symbiotically or non- symbiotically. Addition of nutrients through sedimentation has not been well quantified although evidence suggests that a significant amount of nutrients can be added through this pathway (Piggott et al. 2012).

The calculations involving addition and removal of nutrients in the ecosystem (soil) result in a nutrient balance (Fig 4.1), which can either be negative or positive. Nutrient balances are the differences in nutrient fluxes, which add nutrients to soils (inorganic fertilizer, use of organic manure, biological N fixation, atmospheric deposition - rain, sedimentation during flooding and irrigation water) and nutrient fluxes, which withdraw nutrients from soil (crop products - grains or edible leafy organs, crop residues, leaching, soil erosion and gaseous losses, Stoorvogel et al. 1993).

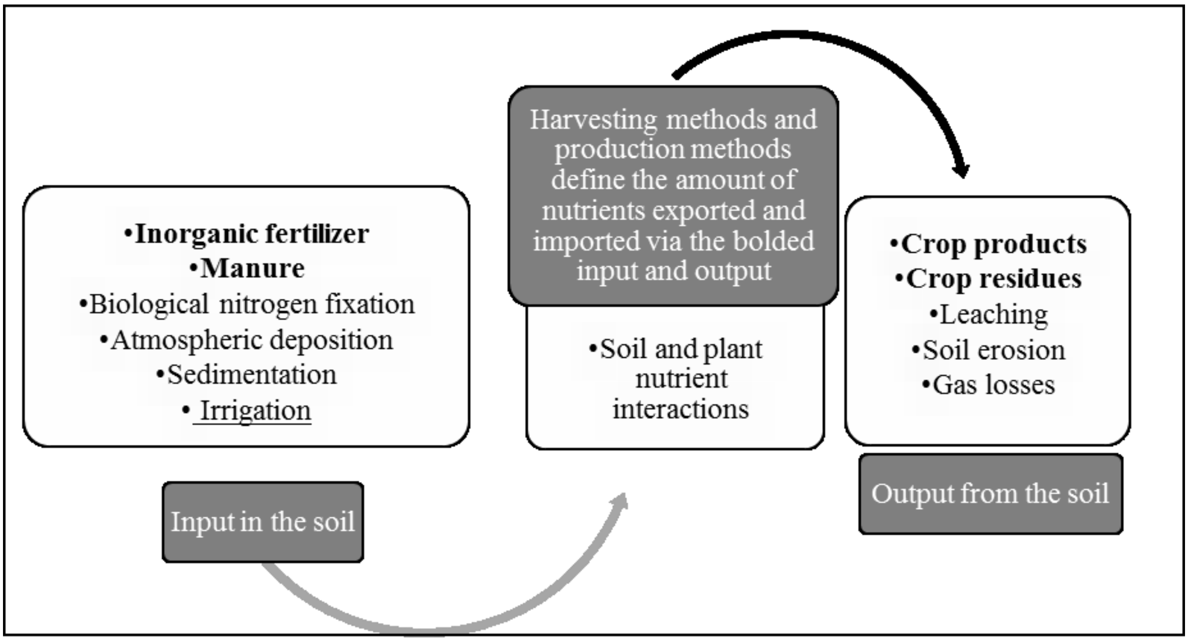

Figure 4.1: Soil nutrients input and output pathways in agricultural production systems.

Among the nutrient fluxes, crop production, crop residues, inorganic fertilizer and manure adding are mainly species-specific and can easily be identified, measured and quantified at field level.

4.2.2 Aims

Most research on AIVs has been done on their importance for human health in terms of vitamins, minerals, proteins and calorie provision. Little or nothing is known about the amount of nutrients (N, P, K, Mg, S and Ca) that are exported from the farm. Available publications on nutrient export focused on N, P, K losses from the soil by cereals. There is less information about the amount of S, Mg and Ca removed by crops grown in sSA and yet these elements have been listed among the limiting nutrients in the soil.

The purpose of this study was to determine the amount of nutrients extracted from the soil by edible organs and crop residues, which are exported by selling the harvest. Of particular interest was the extent to which the species, the production system or the harvesting technique influence the exported quantity of nutrients. Two cropping systems will be compared. In the "batch" system, the crop was completely harvested, clearing the field for the next crop on the same field. In the "continuous" system, at harvest one and two only the edible plant organs were harvested leaving the roots and basal 10 - 15 cm of the shoot in the field. At the third harvest plants were completely harvested. Furthermore, three harvesting methods were compared for the batch system and the last harvest of the continuous system. Plants were either uprooted, cut 5 cm above the soil surface, or only edible organs were harvested. To obtain general data on harvest-related nutrient fluxes, the nutrient enrichment in different plant organs per ton of edible yield is calculated for all species.

When generalized, vegetables are said to have a low nutrient output compared to cereals and pulses (Table 4.1). The determination of the species-specific removal of nutrients by vegetables can help to eliminate the distortion caused by the gerenalization of vegetables. Furthermore, the growth period has to be considered in determining the crop species with high nutrient fluxes.

Table 4.1: Major crops in Kenya and their N, P, K contents (kg ton^{-1}) in harvested organs and crop residues and removal factors (FAO 2004b).

Crop	Harvested product			Crop residues			Removal factor
	N	P	K	N	P	K	
			(kg ton^{-1})				%
Vegetables	9	0.9	2.6	3.2	1.4	7.8	60
Maize	16.8	4.1	4.8	9.7	1.9	21.4	75
Wheat	22.3	4.3	5.8	4.3	1.8	26.7	60
Sorghum	14.5	5.5	3.8	10.8	4.6	29.2	70
Pulses	20	3.4	11.1	10.4	1	13.1	70
Tea	35	3.8	13.4	0.1	0	0	15
Sugar cane	0.6	0.2	1.2	0.3	0.3	0.3	20
Banana	1.2	0.3	4.5	1.6	0.3	11.9	20
Potatoes	4.4	1.3	6.9	2.3	0.7	4.5	20

The aim of the study of AIVs was to find out whether fertilisation recommendations for a cereal plant such as maize are suitable for leafy vegetables and whether the AIVs remove equal amounts of nutrients from the soil. The time of stay and subsequent cycles of planting within a given period determines the amount of soil nutrients exported by a single species. Majumdar et al. (2016), showed that a single crop growth period or cropping cycle determines the amount of nutrients to be applied. It was believed that continuous system could be superior over batch system in terms of minimal soil nutrient export. The information could help farms in choosing the best production system to apply, depending on the fertility of their soils and the vegetable species grown.

4.3 Materials and methods

4.3.1 Study area

A field experiment was conducted at Albrecht Thaer Institute, Dahlem, Berlin - Germany, from June to September 2015. The site lies within latitude 52° 28' N and longitude 13° 18' E. The area receives an average annual temperature of 9.9° C and mean annual rainfall of 562 mm. The field weather data during the study period was as summarized in Table 4.2, while some key physio-chemical soil parameters are shown in Table 4.3.

Table 4.2: Weather information for the months June to September 2015, showing the minimum, average and maximum temperature, relative humidity and precipitation during the time vegetable species were growing on the experimental experiment in berlin.

Month	Average temp (°C)	Maximum temp. (°C)	Minimum temp. (°C)	Relative humidity (%)	NS (mm)
June	17.3	24.8	14.3	65.5	17.7
July	20.1	25.8	15.1	62.4	86.8
August	22.5	29.3	16.2	60.0	30.6
September	14.3	20.6	11.1	75.0	54.8

Table 4.3: Initial soil chemical and physical properties of the field experiment site.

Parameter	pH	Texture	%C	Total content in mg 100 g^{-1} soil		
				N	P	K
Value	6.7	Loamy sand	2:6	n.n.	38	13

4.3.2 Experimental design

The test species comprised of five African indigenous vegetable species *Cleome gynandra* (spider plant), *Vigna unguiculata* (cowpea), *Amaranthus cruentus* (amaranth), *Solanum scabrum* (nightshade) and *Brassica carinata* (Ethiopian kale) and one exotic vegetable, *Brassica olearaceae* (exotic kale). The experiment was laid out as a completely randomized block design, with four blocks and four replicates of six species, respectively. Each plot measured 2 meter length and 1 meter width and a separation pathway of 0.8 m. Two-production systems (batch and continuous) were super- imposed on the study set-up by splitting each plot into two equal parts (Table 4.4).

Table 4.4: Sketchy representation of field experiment plots, where Rep stands for replicate; 1, 2, 3, 4, 5, 6 represent the six vegetable species planted at random; a stands for the batch and b for continuous production system.

Rep. 1	1a	1b	3a	3b	6a	6b	5a	5b	4a	4b	2a	2b
Rep. 2	5a	5b	2a	2b	1a	1b	4a	4b	3a	3b	6a	6b
Rep. 3	4a	4b	5a	5b	2a	2b	6a	6b	1a	1b	3a	3b
Rep. 4	3a	3b	6a	6b	4a	4b	1a	1b	2a	2b	5a	5b

4.3.3 Crop establishment and management

The study site was prepared to a fine tilth mechanically by a tractor plough. To ensure a uniform germination and growth, plant species with low thousand seed weight (amaranth, Ethiopian kale, exotic kale, nightshade and spider plant) were first sown in a greenhouse nursery and later transplanted to the field. To enable a comparable growth, sowing was organized in tiers. Amaranth, African nightshade and spider plant were sown on 2nd of June 2015, while Ethiopian kale and exotic kale were sown on 8th of June 2015. For the greenhouse nursery, three seeds were sown per seed tray compartment. On 25th June, the seedlings (two seedlings per hill) were transplanted from the greenhouse to the field. For cowpea, however, two seeds were sown per hill directly into the field. Two weeks after transplanting/sowing, the plants were thinned to one plant/hill. The field spacing of the species was in accordance to Shackleton et al. (2009). Planting details including days after sowing and spacing are summarized in Table 4.5.

Fertilizer was applied to each plot three days prior to planting. Fertilizers at the rate of 100 kg N ha^{-1} (calcium ammonium nitrate), 100 kg K ha^{-1} (potassium sulfate) and 50 kg P ha^{-1} (triple superphosphate) were applied for amaranth, Ethiopian kale, exotic kale, nightshade and spider plant. Cowpea, being a legume, was only fertilized at the rate of 100 kg K ha^{-1} and 50 kg P ha^{-1}. Nitrogen was not applied to cowpea to encourage nodulation. The experimental plots were kept weed free through manual weeding, while optimum irrigation was done to ensure adequate soil moisture supply to the plants and frequent pest management were undertaken throughout the growing periods to avoid any biotic stress on crop growth.

Table 4.5: Planting time (days) and spacing (cm) of each vegetable species grown in the field experimental site.

Species	Planting	Spacing (cm)	
	DAS	Inter-row	Intra-row
Amaranth	23	20	20
Cowpea	0	50	20
Ethiopian kale	17	50	33
Exotic kale	17	50	33
Nightshade	23	20	20
Spider plant	23	40	20

4.3.4 Harvesting procedures

Only plants from the center of the plots were harvested to ensure that all harvested plants were surrounded by neighbor plants. The plants were harvested when they had reached a size suitable for harvesting according to the guidelines of Shackleton et al. (2009). Plant age at harvest in days after sowing (DAS) and days after transplanting to the field (DAP) are given in Table 4.6.

For the batch system, the plants were pulled out of the soil, so the whole shoot with coarse roots adhering to the shoot were harvested. After harvest from the field, the plants were divided into coarse roots, basal 5 cm of the shoot ("stubble") and leaves including petioles, laterals and stems.

For the continuous system, three harvests were done. At the first and second harvest, only the young parts of the shoot were harvested. This was done by cutting all shoots at about 10 cm above the soil surface. After harvest, the plants were separated into leaves plus young laterals (considered as "edible" plant organs), and stems. At the third (final) harvest, plants were pulled out of the soil, as for the batch system. After harvest, the plants were separated into leaves plus young laterals, stems, stubbles (basal 5 cm of the shoot), and coarse roots.

Table 4.6: Number of harvested plants per plot and cultivation period until harvest in days after sowing (DAS) and days after planting (DAP) for batch and continuous systems for the field experiment in Berlin. (TP-H1: Transplanting to harvest 1; H1-H2: Harvest 1 to harvest 2; H2-H3: Harvest 2 to harvest 3).

	No.of harvested plants	Cultivation Period (days)		Harvest time (days)		
		DAS	DAP	TP- H1	H1-H2	H2-H3
		Batch production system				
Amaranth	8	57	34	34	-	-
Cowpea	12	47	47	47	-	-
Ethiopian kale	8	51	34	34	-	-
Exotic kale	8	51	34	34	-	-
Nightshade	8	57	34	34	-	-
Spider plant	12	57	34	34	-	-
		Continuous production system				
Amaranth	24	111	88	34	21	33
Cowpea	15	87	87	48	16	23
Ethiopian kale	10	105	88	34	15	39
Exotic kale	10	105	88	34	19	26
Nightshade	27	111	88	34	21	33
Spider plant	18	111	88	34	21	33

4.3.5 Laboratory analysis

All fresh plant samples were washed with distilled water to remove soil and dust adhering to the surface. Then, fresh mass and dry mass (drying at 70°C to constant mass) were determined. Dried plant samples were milled and stored in plastic bottles for element analysis. Sample digestion and analysis for N, P, K, S, Ca, Mg and Zn was done as prescribed in paragraph 3.3.7 of the chapter 3.

4.3.6 Calculations and Statistics

For the batch system, cowpea grew 47 days, all other plants grew for 34 days in the field, whereas for the continuous system, cowpea grew 87 days and all other plants grew for 88 days in the field. To compare yield and nutrient withdrawal in the two systems, we assumed that 88 (87) days for the batch system were equivalent to 2.5 cropping seasons (from transplanting to harvest). Thus, we calculated the data for the second and third batch crop yield on the basis of the data of the first crop yield. For that case, we assumed that under the prevailing growing conditions, i.e. supply of water and mineral nutrients, plant growth would only depend on temperature. We determined the actual temperature sum (degree days) during the first, second, and third growth cycle, which was actually imposed on the plants of the continuous system (Table 4.7). Then we calculated species-specific coefficients, which were used for calculation of the biomass for plants that would have grown in a batch system in the second and third growth cycle (Table 4.8). With this coefficients and the yield of harvest 1 in the batch system we calculated the potential yield at harvest 2 and 3.

Table 4.7: Temperature sums (degree days) during the first, second, and third growth cycle of AIVs in continuous production system. In batch system AIVs were harvested once at harvest 1.

Species	Harvest 1		Harvest 2		Harvest 3	
	Batch	Continuous	Batch	Continuous	Batch	Continuous
	Growth duration in degree days					
Amaranth	709	709	0	464	0	579
Cowpea	1008	1008	0	343	0	401
Ethiopian kale	709	709	0	347	0	696
Exotic kale	709	906	0	403	0	443
Nightshade	709	709	0	464	0	579
Spider plant	709	709	0	464	0	579

Table 4.8: Species-specific coefficients used to calculate the expected yields for harvests 2 and 3 in the batch system. Coefficients for harvest 2 are the ratios of degree-days during second and first growth period, Coefficients for harvest 3 are the ratios of degree-days during third and first growth period.

Species	Harvest 1	Harvest 2	Harvest 3
	Species-specific coefficients		
Amaranth	1	0.65	0.82
Cowpea	1	0.34	0.40
Ethiopian kale	1	0.49	0.98
Exotic kale	1	0.44	0.49
Nightshade	1	0.65	0.82
Spider plant	1	0.65	0.82

Yield data

At harvest, the yields of plant fresh and dry mass were determined in grams per unit area harvested (m^2). Since the absolute unit harvest areas varied across the species, the fresh and dry mass yields were converted to tons per hectare as detailed below.

Since, 1 g = 0.001kg or 0.000001 tons, and 1 m^2 = 0.0001 hectare,

Therefore, X yield in g/m^2 = (X * 0.000001/0.0001) tons/ ha

4.3.7 Data analysis

R statistical software was used for means and standard deviation determination. The residuals were tested for normality using q-q plots, histogram and density mean. Homogeneity of variances was tested using Bartlett's test for equal variance. One and two way analysis of variance (ANOVA) was used to find out the significant differences ($p < 0.05$) among the plant species, followed by a post hoc LSD test for mean separation. Paired T-test ($p < 0.05$) was used to determine differences in nutrient removal among vegetable species between the production systems.

4.4 Results

4.4.1 Yield of edible plant organs

Leaf fresh mass, which was considered to be an appropriate measure for the yield of edible organs, significantly differed among species and production systems (Table 4.9). In the batch production system, the yield at harvest 1 varied between 33.6 x 10^3 kg ha^{-1} for Ethiopian kale and 14.1 x 10^3 kg ha^{-1} for cowpea. As the yields for harvests 2 and 3 were calculated on the basis of the yield for harvest 1, the yields of the different species for these harvests, and total yield (sum of harvests 1 to 3) reflected the differences found for harvest 1. In the continuous production system, the yield at harvest 1 varied between 25.4 x 10^3 kg ha^{-1} for exotic kale and 9.3 x 10^3 kg ha^{-1} for cowpea.

The yield differences among species and the ranking of species do not exactly correspond to those found for the batch system, because the contribution of leaves in 0 - 10 cm plant height, which were not harvested in the continuous system, varied among species. In the continuous system, the yields at harvest 2 were larger than at harvest 1 despite of the lower temperature sum acting on the plants in the second period (343 - 464 degree days) in comparison to the first period (709 degree days, except for cowpea and exotic kale) (Table 4.7).

Table 4.9: Fresh mass (10^3 kg ha^{-1}) of edible organs (leaves and laterals) of vegetable species (mean ± standard deviation) in batch and continuous production system at three harvests. Columns with same lower case letters behind the means indicate no significant differences between means of production systems of a particular species, rows with same upper case letters behind the means indicate no significant differences among means of harvests of a particular species ($p > 0.05$, ANOVA, LSD test, $n = 4$) ($p > 0.05$, ANOVA, LSD test, $n = 4$). Yield for harvests 2 and 3 in batch production sytem were calculated from yields of harvest 1 and coefficients of table 4.8; XK: exotic kale, EK: Ethiopian kale, NS: African nightshade, AM: amaranth, CP: cowpea, SP: spider plant.

Species	Production	Harvesting period			
		Harvest 1	Harvest 2	Harvest 3	Total
		Edible fresh mass (10^3 kg per ha)			
XK	Batch	20.3 ± 3.4 aA	8.9 ± 1.5 bB	9.9 ± 1.7 bB	39.2 ± 6.5 b
	Continuous	25.4 ± 7.8 aA	31.5 ± 5.2 aA	21.9 ± 1.3 aA	78.8 ± 3.8 a
EK	Batch	33.6 ± 4.4 aA	16.5 ± 2.2 bB	33.0 ± 4.3 aA	83.1 ± 10.9 a
	Continuous	13.6 ± 0.9 bB	20.0 ± 4.7 aAB	25.5 ± 6.4 aA	59.1 ± 5.4 b
NS	Batch	28.9 ± 4.4 aA	18.8 ± 2.0 aC	23.7 ± 2.6 aB	71.4 ± 7.8 a
	Continuous	14.3 ± 1.8 bB	22.4 ± 3.0 aA	13.7 ± 2.4 bB	50.4 ± 4.5 b
AM	Batch	19.9 ± 3.1 aA	12.9 ± 2.0 bB	16.3 ± 2.5 aAB	49.2 ± 7.6 a
	Continuous	11.9 ± 0.8 bB	20.1 ± 2.0 aA	12.5 ± 2.1 aB	44.4 ± 4.0 a
CP	Batch	14.1 ± 3.8 aA	4.8 ± 1.3 bB	5.6 ± 1.5 bB	24.5 ± 6.6 a
	Continuous	9.3 ± 1.0 aA	9.7 ± 1.7 aA	2.8 ± 0.6 aB	21.9 ± 2.7 a
SP	Batch	18.5 ± 2.3 aA	12.0 ± 1.5 aC	15.2 ± 1.9 aB	45.7 ± 5.8 a
	Continuous	12.7 ± 3.6 bA	15.2 ± 5.1 aA	8.8 ± 4.5 aA	36.7 ± 12.3 a

The higher yields in the second period in continuous production system are presumably due to the fact that the plants started into the second period with a well-developed root system and further reserves contained in the shoot base. Accordingly, the time needed to reach nearly complete soil cover by foliage was lower in the second, than in the first period (Marlene Bittner, Master-thesis 2017). The yields at harvest 3, in tendency, decreased again (except for Ethiopian kale). This was possibly due to the higher physiological age of plants, which was associated with increased biomass allocation to reproductive plant organs (flowers, berries, seeds).

The production system influenced total yield in a species-specific way. For Ethiopian kale and African nightshade, the total yield was significantly higher in the batch system than in the continuous system (Table 4.9). However, exotic kale's total yield in continuous system was significantly higher than batch system due to much less yield produced in harvest 2 and 3 as a result of late harvesting in harvest 1. For the other three species, there were no significant effects of the production system on total yield, whereby, in tendency, their total yields were higher in the continuous, than in the batch system. These species-specific differences in the yield response to the production system may possibly reflect differences among species in physiological ageing and ability for re-sprouting after cutting. Presumably, the response of species to the production system and harvesting periods is dependent on the specific environmental conditions, e.g. day lengths and air temperatures, and thus is site-specific.

4.4.2 Biomass partitioning between edible organs and non-edible organs

From a nutritional point of view, total plant biomass of leafy vegetables can be divided into edible organs (leaves) and non-edible organs (rest of the plant). From a horticultural point of view, non-edible organs can be further divided into organs, which are sold in the market together with the leaves and organs, which remain in the field or are used within the farm (e.g. as feed or fuel). The ratio of organs sold in the market is dependent on the harvest technique (Fig.4.2). The organs sold in the market include leaves, stems and coarse roots for plants harvested by pulling them out of the soil, total shoots except the basal part for plants harvested by cutting, and leaves only for the harvest technique "picking".

Figure 4.2: Plant organs that are transported to the market and those left in the soil (as shown by the black line) depending on the harvest technique. The organs above the black lines are those exported to the markets while those below the line are imported into the soil.

In our study, we quantified the biomass of different organs separately to assess the effects of vegetable species, harvest technique, and production system on the ratio of total plant biomass sold in the market. Fine roots were not considered, because most of them remain in the soil when the crops are pulled. Leaves made up the largest portion of the separated plant organs in all vegetables in the batch system, ranging between 2.1 x 10^3 kg ha^{-1} for cowpea and 8.8 x 10^3 kg ha^{-1} for Ethiopian kale (Table 4.10). On the other hand, spider plant produced similar amount of leaves and stem dry mass. Amaranth and spider plant produced the highest amount of stem dry mass while stem yields of cowpea and exotic kale were significantly lower. The amount of dry mass produced in the coarse roots was equivalent to the mass of stubbles for all the species. The stubbles were the part of the stem left after cutting the plants at 5 cm height from ground. Amaranth had significantly higher stubbles and coarse roots biomass compared to other vegetables species.

Table 4.10: Dry mass (10^3 kg ha^{-1}) of various plant organs in batch production system (mean ± standard deviation). Data are means of the sum of all three harvests for all plant organs. Means marked with same lower case letters within a column indicate no significant differences among means of species, means with same upper case letters within rows indicate no significant differences among means of plant organs ($p > 0.05$, ANOVA, LSD test, n = 4). *Coarse roots and stubbles were bulked for the analysis.

Species	Plant organ			
	Leaves	Stems	Stubbles	Coarse roots
	Dry mass (10^3 kg ha^{-1})			
Exotic kale	4.2 ± 0.64 cA	0.47 ± 0.09 cB	0.33 ± 0.12 dB	0.14 ± 0.04 cB
Ethiopian kale	8.8 ± 1.46 aA	2.87 ± 0.78 bB	1.06 ± 0.19 bC	0.20 ± 0.02 cC
African nightshade	5.6 ± 0.61 bA	2.25 ± 0.35 bB	0.62 ± 0.16 cC	0.57 ± 0.08 aC
Amaranth	6.5 ± 0.80 bA	5.28 ± 0.82 aB	1.30 ± 0.04 aC	0.66 ± 0.07 aC
Cowpea	2.1 ± 0.50 dA	0.41 ± 0.08 cB	nd	0.22 ± 0.06 cB*
Spider plant	6.3 ± 1.46 bA	5.65 ± 0.88 aA	0.79 ± 0.20 cB	0.42 ± 0.13 bB

In case of continuous system, all species obtained the highest dry mass in the leaves compared to other plant organs, apart from spider plant, that had similar dry mass with its stem (Table 4.11). The kales obtained the highest (8.4 and 7.5 x 10^3 kg ha^{-1}) leaf dry mass and cowpea the least (3.4 x 10^3 kg ha^{-1}) leaf dry mass compared with other species. In continuous system, coarse roots had the lowest dry mass from all the other organs ranging between 0.10 x 10^3 kg ha^{-1} for cowpea and 0.72 x 10^3 kg ha^{-1} for amaranth. Unlike in the batch system, the stubbles of all the vegetable species had significantly higher dry mass than the coarse roots. Amaranth had the largest stubble and coarse root dry mass, compared with other species. In this production system, the stubbles grew bigger in order to provide better support to the stems, laterals and many branching of the leaves canopy and that is the reason why some species like exotic kale and amaranth had significantly higher stubble dry mass compared to the stems, whereas the stem and stubble dry mass for cowpea and African nightshade were similar.

Table 4.11: Dry mass (10^3 kg ha^{-1}) of various plant organs in continuous production system (mean ± standard deviation). Data for Leaves and stems are means of the sum of all three harvests, while for stubble, coarse and fine roots data are means for harvest 3. Means marked with same lower case letters within a column indicate no significant differences among means of species, means with same upper case letters within rows indicate no significant differences among means of plant organs (p > 0.05, ANOVA, LSD test, n = 4).

Species	Plant organ			
	Leaves	Stems	Stubbles	Coarse roots
	Dry mass (10^3 kg ha^{-1})			
Exotic kale	8.4 ± 0.40 aA	0.66 ± 0.16 dC	1.45 ± 0.18 cB	0.29 ± 0.05 bcC
Ethiopian kale	7.5 ± 0.39 aA	6.88 ± 0.56 aB	2.23 ± 0.30 bC	0.47 ± 0.06 bD
African nightshade	5.2 ± 0.35 cA	1.61 ± 0.12 dB	1.92 ± 0.22 bcB	0.48 ± 0.03 bC
Amaranth	6.4 ± 0.21 bA	2.71 ± 0.42 cC	4.91 ± 0.30 aB	0.72 ± 0.27 aD
Cowpea	3.4 ± 0.45 dA	0.88 ± 0.10 dB	0.65 ± 0.18 dB	0.10 ± 0.03 cC
Spider plant	4.7 ± 1.44 cA	4.51 ± 1.39 bA	2.35 ± 0.62 bB	0.39 ± 0.05 bC

4.4.3 Nitrogen concentration in different organs

Mineral concentrations in plants differ according to the organs, as plant minerals are majorly translocated from the roots up to the leaves or plant reproductive organs. The trend in which nutrients accumulate in plants differs due to their mobility. In order to avoid repetitions in the description of the results, we have decided to present the results for the N concentration in the harvested plant organs of the studied vegetable species as a representative of other measured mineral concentrations.

For the batch system, the concentration of N was higher in the leaves than the other organs of the plant ranging from 29 g N kg^{-1} dry mass for cowpea to 52 g N kg^{-1} dry mass for spider plant (Table 4.12). It should be noted that the concentration of P, K, S, Mg and Ca (g kg^{-1} dry mass) in the leaves differed among the vegetable species and did not follow the same order as it appears for N (not shown in the table). The concentration of N was high in the leaves for the fact that leaves play a major role for physiological functions and thus require nutrients for effective functioning. Most minerals usually end up in leaves unless the plants are entering reproductive stage, where minerals are translocated to flowers and seeds/fruits. Furthermore, nutrient deficiency might result in nutrient re-translocation out of leaves. Some plant activities take place in the stems as well and that is why stems were second largest reservoirs of N in most vegetable species apart from amaranth, whose concentrations in the stem, stubbles, course roots and fine roots were similar.

Table 4.12: Nitrogen concentration (g kg^{-1} dry mass) in plant organs of vegetable species under batch production system (mean ± standard deviation). Means marked with same lower case letters within a column indicate no significant differences among means of species, means with same upper case letters within rows indicate no significant differences among means of plant organs ($p > 0.05$, ANOVA, LSD test, $n = 4$).

Species	Plant organ				
	Leaves	Stem	Stubbles	Coarse roots	Fine roots
	Nitrogen concentration (g kg^{-1} dry mass)				
Exotic kale	49 ± 3 aA	38 ± 4 aB	21 ± 1 aCD	25 ± 3 aC	19 bD
Ethiopian kale	40 ± 2 bA	23 ± 5 bB	19 ± 3 abC	21 ± 1 bBC	8 fD
African nightshade	42 ± 7 bA	26 ± 2 bB	12 ± 1 cC	13 ± 1 cC	14 dC
Amaranth	31 ± 3 cA	15 ± 2 cB	16 ± 3 bB	17 ± 2 cB	15 cB
Cowpea	29 ± 5 cA	16 ± 4 cBC	nd	14 ± 2 cC	20 aB
Spider plant	52 ± 4 aA	24 ± 6 bB	17 ± 2 bCD	20 ± 3 bBC	11 eD

Nitrogen concentrations were at intermediate levels in stubbles and coarse roots, while fine roots had significantly lower concentrations in both kale species and spider plant. African nightshade showed similar N concentrations in stubbles, coarse and fine roots. In amaranth similar N concentrations in stems, stubbles and the root fractions were found. Interestingly, cowpea had higher N concentrations in fine roots than in the course roots. Among all species, exotic kale had the highest levels of N in all the plant organs, exept fine roots, while amaranth and cowpea had the lowest N concentrations in most of their plant organs.

The continuous system was roughly similar to the batch system, where the leaves had the highest N concentrations in all vegetables species, although the high nitrogen concentrations in leaves could be found in stems of exotic kale as well (Table 4.13). The N concentrations in leaves ranged from 27 g kg^{-1} dry mass for cowpea to 47 g kg^{-1} dry mass for spider plant. The concentration of N in stems was significantly higher than in the stubbles and coarse roots in all vegetable species. For the case of this production system, fine roots of cowpea, amaranth and spider plant had similar N concentrations as stems. The N concentrations in the stem ranged from 16 g kg^{-1} dry mass for amaranth to 40 g kg^{-1} dry mass in exotic kale, whereas stubbles had N concentration ranges of 9 g kg^{-1} dry mass for African nightshade and amaranth to 20 g kg^{-1} dry mass for exotic kale. The concentration range for coarse roots was slightly lower than for stubbles (8 – 15 g kg^{-1} dry mass), nevertheless, there were no significant differences among vegetables species. Fine roots showed a typical N concentration range of between 13 g kg^{-1} dry mass for African nightshade and amaranth to 21 g kg^{-1} dry mass for exotic kale.

Table 4.13: Nitrogen concentration (g kg^{-1} dry mass) in plant organs in the continuous production system (mean ± standard deviation). Data for Leaves and stems are means for all three harvests, while for stubble, coarse and fine roots data are means for harvest 3. Means marked with same lower case letters within a column indicate no significant differences among means of species, means with same upper case letters within rows indicate no significant differences among means of plant organs ($p > 0.05$, ANOVA, LSD test, n = 4).

Species	Plant organ				
	Leaves	Stem	Stubbles	Coarse roots	Fine roots
	Nitrogen concentration (g kg^{-1} dry mass)				
Exotic kale	39 ± 3 bA	40 ± 7 aA	20 ± 2 aBC	15 ± 4 aC	21 aB
Ethiopian kale	30 ± 2 cdA	23 ± 2 bcB	10 ± 3 bD	10 ± 4 aD	15 bC
African nightshade	33 ± 2 cA	25 ± 1 bB	9 ± 1 bD	10 ± 3 aCD	13 bC
Amaranth	32 ± 2 cdA	16 ± 1 dB	9 ± 1 bCD	8 ± 5 aD	13 bBC
Cowpea	27 ± 4 dA	19 ± 3 cdB	12 ± 2 bC	13 ± 3 aC	19 aB
Spider plant	47 ± 4 aA	21 ± 2 bcB	12 ± 2 bC	12 ± 2 aC	18 aB

4.4.4 Effects of production systems and harvesting techniques on nutrient fluxes from soil to market

As described in section 4.3.2, farmers apply different techniques in harvesting their crops. Most farmers prefer the batch system, where they terminate the crops as soon as they attain the harvesting stage. The common harvesting method preferred by many farmers is pulling and to some small extent cutting, because these methods are less time consuming.

Unfortunately, farmers are not aware of the amount of nutrients that go alongside the harvested plant organs. In our experiment, we quantified the amount of nutrients that is removed by these three harvesting techniques in both production systems.

4.4.4.1 Effects of production systems and harvesting techniques on N removal

Vegetable species, harvested by picking exported less N to the market as opposed to cutting and pulling harvesting system (Table 4.14). This was expected because only a small portion of the plant was removed from the farm. The exportation of N from the soil ranged from 3.0 kg per 10^3 kg leaf fresh mass for African nightshade harvested by picking to 9.9 kg per 10^3 kg leaf fresh mass for spider plant harvested by pulling. Considering picking of edible organs, there were no differences in the amount of N removed between the batch and continuous system in all vegetables. In both harvesting techniques, spider plant accumulated the highest levels of N in its leaves and cowpea the lowest compared to other species. It was only Ethiopian kale that showed significant differences between the batch and continuous production system by removing highest amount of N from the soil to the market in the continuous system. Pulling of vegetables led to Ethiopian kale and African nightshade exporting more N in the continuous system than batch, while the other species did not show any significant difference between production systems.

The production system had little impact/effect on the amount of N removed from the soil since few species showed significant differences. On the other hand, there were pronounced species differences in the N removed per kg fresh mass, whereby spider plant would remove remarkably more N per kg fresh mass from the soil and African nightshade the least amount of N per kg fresh mass.

Table 4.14: Yield-based nitrogen removal (kg per 10^3 kg leaf fresh mass) with harvested plant organs from soil to market as affected by species, production system and harvest method (mean ± standard deviation). Means with the same lower case letters within the columns show no significant difference between the two production systems for each of the harvest methods analysed, means with the same upper case letters within rows indicate no significant differences among species ($p > 0.05$, ANOVA, LSD test, $n = 4$).

Production system	Species					
	Exotic kale	Ethiopian kale	African nightshade	Amaranth	Cowpea	Spider plant
	N removal (kg per 10^3 kg leaf fresh mass)					
	Picking					
Batch	4.4 ± 0.34 aB	3.5 ± 0.34 aCD	3.0 ± 0.43 aD	4.2 ± 0.22 aB	3.9 ± 0.41 aBC	6.5 ± 0.50 aA
Continuous	4.2 ± 0.30 aBC	3.7 ± 0.17 aBC	3.5 ± 0.34 aC	4.5 ± 0.31 aB	4.2 ± 1.03 aBC	6.2 ± 0.30 aA
	Cutting					
Batch	4.8 ± 0.32 aC	4.3 ± 0.56 bCD	3.8 ± 0.45 aD	5.8 ± 0.33 aB	4.2 ± 0.46 aCD	9.5 ± 0.99 aA
Continuous	4.5 ± 0.41 aCD	5.9 ± 0.55 aB	4.3 ± 0.32 aD	5.5 ± 0.32 aBC	5.0 ± 1.19 aBC	8.7 ± 0.43 aA
	Pulling					
Batch	5.1 ± 0.31 aC	4.6 ± 0.55 bCD	4.0 ± 0.46 bD	6.5 ± 0.30 aB	4.3 ± 0.46 aCD	9.9 ± 1.10 aA
Continuous	4.9 ± 0.41 aD	6.3 ± 0.61 aBC	4.7 ± 0.33 aD	6.7 ± 0.47 aB	5.4 ± 1.30 aCD	9.6 ± 0.48 aA

Species relative N export values were calculated relative to the batch production system under picking harvesting technique (Fig. 4.3). Exotic kale showed no big differences in N export among the three harvesting methods. In all remaining species, little differences existed between continuous system harvested by picking and the standard measure for all the species. Ethiopian kale, African nightshade, amaranth and spider plant removed over 1.2 times more N under cutting and pulling in both production systems, with Ethiopian kale removing the highest amounts of N, of about 1.7 and 1.8 times more than the standard system when continuously produced. Cowpea grown in continuous production system, harvested by cutting and pulling, removed 1.2 times more N as in batch system.

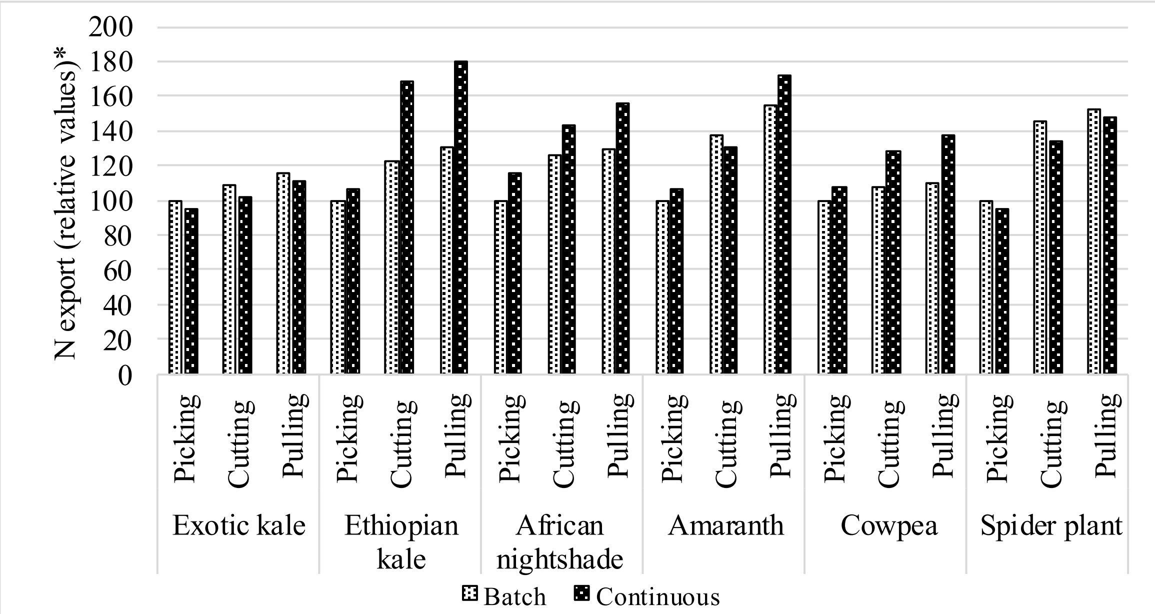

Figure 4.3: Effect of harvest technique (cutting, picking, pulling) and production system (batch, continuous) on N export from soil to market in different vegetable species; *for each species, the N export in the batch system harvested by picking was set to 100%.

4.4.4.2 Effects of production systems and harvesting techniques on P removal

The amount of P exported from the soil to the market was dependant on, harvesting techniques and production systems (Table 4.15). The P export ranged from 0.54 kg per 10^3 kg leaf fresh mass for African nightshade grown in the batch system and harvested by picking to 2.76 kg per 10^3 kg leaf fresh mass for amaranth grown in the continuous system and harvested by cutting the crop. African nightshade and cowpea removed significantly higher amounts of P in the continuous system than in the batch system when harvested by picking. When cutting edible organs, Ethiopian kale, African nightshade and cowpea showed significantly higher P removal in the continuous system as well. For pulling, more crops showed differences between the production systems, whereby all species, apart from spider plant, removed significantly more P from the soil under continuous system. Spider plant exported the highest P amounts to the market among all the species in both production systems with picking and cutting, whereas with pulling, under continuous system, amaranth removed the highest amount of P of all species. In comparison to other species, exotic kale exported the lowest amount of P, when crops were harvested by cutting and pulling. The characteristic morphology of exotic kale with small stem, stubble and coarse roots (Table 4.10 and 4.11) could have played a role for the low P accumulation.

Considering the relative values, more than twice of the P removed in the standard system was removed in Ethiopian kale grown in the continuous system and harvested by cutting and pulling as well as in amaranth harvested by pulling (Fig 4.4). Cowpea showed an increase of 30 % in export of P in continuous system harvested by picking, whereas the P removal just slightly increased from the standard system in all other species except, spider plant, which showed a weak decrease in P export.

The amounts of P exported by Ethiopian kale, amaranth and spider plant in the batch system were more than 1.4 times higher, when harvested by cutting or pulling in comparison to picking. Thus, in all the vegetable species, cutting and pulling harvesting technique removed more P than N compared with picking.

Table 4.15: Yield-based phosphorous removal (kg per 10^3 kg leaf fresh mass) with harvested plant organs from soil to market as affected by species, production system and harvest method (mean ± standard deviation). Means with the same lower case letters within the columns show no significant difference between the two production systems for each of the harvest methods analysed, means with the same upper case letters within rows indicate no significant differences among species ($p > 0.05$, ANOVA, LSD test, n = 4).

Production system	Exotic kale	Ethiopian kale	African nightshade	Amaranth	Cowpea	Spider plant
			P removal (kg per 10^3 kg leaf fresh mass)			
			Picking			
Batch	0.57 ± 0.01 aC	0.55 ± 0.08 aC	0.54 ± 0.01 bC	0.92 ± 0.04 aB	0.58 ± 0.02 bC	1.41 ± 0.13 aA
Continuous	0.59 ± 0.02 aD	0.61 ± 0.03 aD	0.62 ± 0.04 aD	0.96 ± 0.05 aB	0.75 ± 0.10 aC	1.31 ± 0.04 aA
			Cutting			
Batch	0.65 ± 0.01 aD	0.79 ± 0.09 bC	0.72 ± 0.03 bCD	1.86 ± 0.14 aB	0.63 ± 0.03 bD	2.21 ± 0.04 aA
Continuous	0.64 ± 0.01 aF	1.24 ± 0.10 aC	0.81 ± 0.04 aE	1.49 ± 0.04 bB	0.94 ± 0.11 aD	2.02 ± 0.10 bA
			Pulling			
Batch	0.73 ± 0.01 bCD	0.87 ± 0.09 bC	0.79 ± 0.03 bCD	2.19 ± 0.18 bB	0.65 ± 0.03 bD	2.38 ± 0.12 aA
Continuous	0.78 ± 0.01 aE	1.44 ± 0.12 aC	1.06 ± 0.03 aD	2.76 ± 0.17 aA	1.06 ± 0.14 aD	2.40 ± 0.12 aB

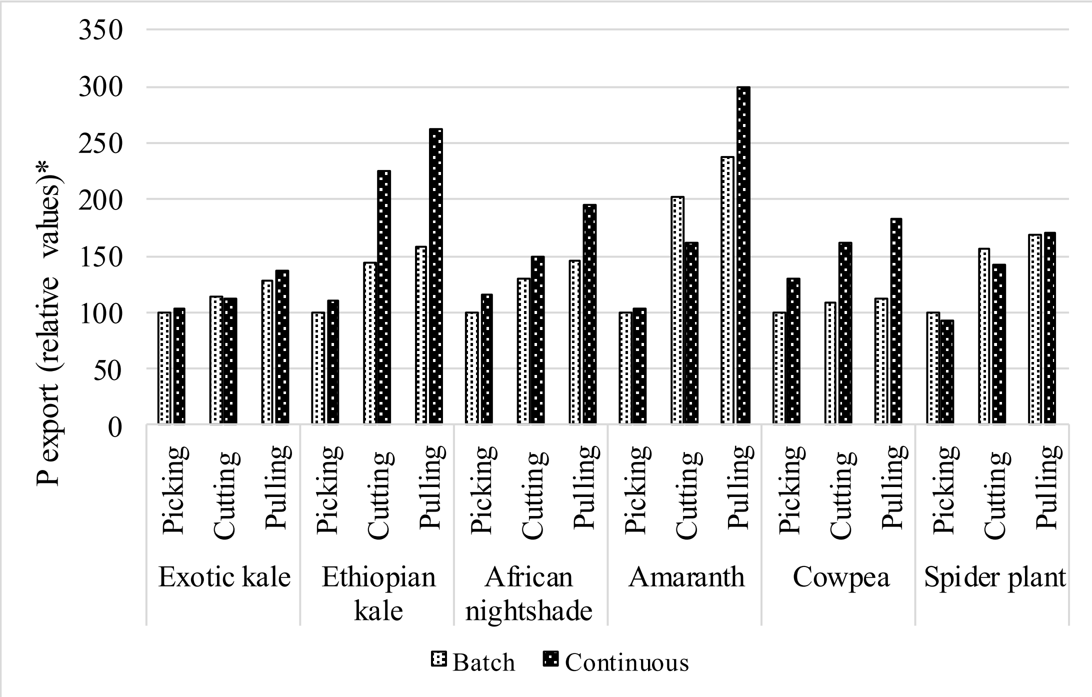

Figure 4.4: Effect of harvest technique (cutting, picking, pulling) and production system (batch, continuous) on P export from soil to market in different vegetable species; *for each species, the N export in the batch system harvested by picking was set to 100%.

4.4.4.3 Effects of production systems and harvesting techniques on K removal

Vegetable species differed in the amount of K exported to the market, ranging from 4.4 kg per 10^3 kg leaf fresh mass for cowpea grown in batch system, harvested by picking to 18.9 kg per 10^3 kg leaf fresh mass for amaranth grown in continuous system and harvested by pulling (Table 4.16). Most species did not have significant differences in K removal between the production systems when harvested by picking apart from African nightshade that exported more K in the continuous system than in batch system.

Table 4.16: Yield-based potassium removal (kg per 10^3 kg leaf fresh mass) with harvested plant organs from soil to market as affected by species, production system and harvest method (mean ± standard deviation). Means with the same lower case letters within the columns show no significant difference between the two production systems for each of the harvest methods analysed, means with the same upper case letters within rows indicate no significant differences among species ($p > 0.05$, ANOVA, LSD test, $n = 4$).

Production system	Species					
	Exotic kale	Ethiopian kale	African nightshade	Amaranth	Cowpea	Spider plant
	K removal (kg per 10^3 kg leaf fresh mass)					
	Picking					
Batch	4.9 ± 0.09 aCD	5.1 ± 0.51 aBC	4.6 ± 0.09 bDE	7.2 ± 0.18 aA	4.4 ± 0.26 aE	5.4 ± 0.33 aB
Continuous	5.0 ± 0.35 aB	5.2 ± 0.21 aB	5.1 ± 0.11 aB	7.5 ± 0.29 aA	5.0 ± 1.41 aB	4.7 ± 0.17 bB
	Cutting					
Batch	5.5 ± 0.05 aDE	7.1 ± 0.79 bC	6.3 ± 0.82 aCD	14.9 ± 0.90 aA	5.1 ± 0.27 aE	10.8 ± 0.54 aB
Continuous	5.5 ± 0.42 aD	9.0 ± 0.55 aB	7.2 ± 0.17 aC	11.9 ± 0.28 bA	6.7 ± 1.46 aC	9.4 ± 0.30 bB
	Pulling					
Batch	5.9 ± 0.12 aDE	7.6 ± 0.84 bC	6.9 ± 0.78 bCD	17.4 ± 1.02 aA	5.4 ± 0.27 bE	12.0 ± 0.81 aB
Continuous	6.5 ± 0.38 aE	10.2 ± 0.84 aC	9.3 ± 0.41 aCD	18.9 ± 0.56 aA	8.2 ± 1.67 aD	11.8 ± 0.41 aB

Species varied in the amount of K exported when harvested by picking with amaranth exporting the highest K amounts of all the species. Harvesting of vegetables by cutting resulted in Ethiopian kale exporting significantly higher amounts of K in the continuous system. However, amaranth and spider plant exported larger amounts of K in the batch system compared to continuous system. Pulling of vegetables increased K export in the continuous system by Ethiopian kale, African nightshade and cowpea. Regardless of harvest technique or production system, amaranth exported always highest amounts of K among species. Exotic kale always exported the least amounts of K in continuous system and together with cowpea least quantities of K in batch system as well.

The relative values calculated from table 4.16 showed that amaranth and spider plant harvested by pulling are likely to export more than 2 times more K than when the species were picked (Fig. 4.5). In the continuous system, Ethiopian kale, African nightshade, cowpea and spider plant tentatively removed above 1.5 times more of K when harvested by cutting and pulling as compared with the standard measure. In the batch system, most species did not exceed 1.5 times the amount of K when harvested by cutting and pulling, compared with the standard, apart from amaranth and spider plant.

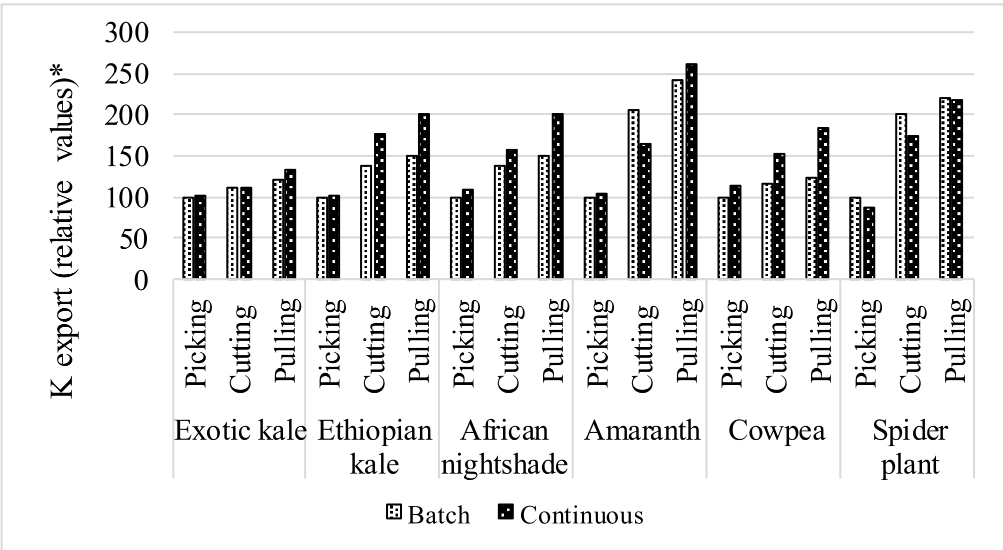

Figure 4.5: Effect of harvest technique (cutting, picking, pulling) and production system (batch, continuous) on K export from soil to market in different vegetable species; *for each species, the N export in the batch system harvested by picking was set to 100%.

When picking edible plant organs, K export in most species is hardly influenced by the production system. As it was observed with N and P, exotic kale exported the lowest amount of K among all the vegetable species when pulled.

4.4.4.4 Effects of production systems and harvesting techniques on S removal

Vegetable species also varied in the amount of S they exported to the market with a range between 0.36 kg per 10^3 kg leaf fresh mass for cowpea harvested by picking leaves in the batch production system and 2.86 kg per 10^3 kg leaf fresh mass for Ethiopian kale harvested by pulling in the continuous production system (Table 4.17). Picking of leaves during harvest resulted in Ethiopian kale, African nightshade and amaranth exporting more S in the continuous system than in the batch system. Among all species, the kales exported highest amounts of S to the market and cowpea exported least. When the species were harvested by cutting, exotic kale, Ethiopian kale and spider plant exported highest amounts of S in the continuous system as well. In the continuous system, pulling of leafy vegetables resulted in a higher S export for all species than in the batch system.

The kales and spider plant exported more S than other species when harvested by cutting and pulling in the batch system, while only Ethiopian kale removed significantly higher S in the continuous system. Cutting and pulling of Cowpea exported the least S compared to all other vegetable species.

Considering the relative values, cutting and pulling of Ethiopian kale and pulling amaranth, cowpea and spider plant, exported more than twice the amount of S in the continuous system (Fig. 4.6). The amount of S removed by pulling Ethiopian kale, amaranth and cowpea in the batch system was almost half that of the continuous system. Among all vegetable species, amaranth and spider plant exported more than 1.5 times the amount of S when harvested by cutting and pulling. Exotic kale and African nightshade exported similar amounts of S among the three harvesting systems.

Table 4.17: Yield-based sulphur removal (kg per 10^3 kg leaf fresh mass) with harvested plant organs from soil to market as affected by species, production system and harvest method (mean ± standard deviation). Means with the same lower case letters within the columns show no significant difference between the two production systems for each of the harvest methods analysed, means with the same upper case letters within rows indicate no significant differences among species ($p > 0.05$, ANOVA, LSD test, n = 4).

Production system	Species					
	Exotic kale	Ethiopian kale	African nightshade	Amaranth	Cowpea	Spider plant
	S removal (kg per 10^3 kg leaf fresh mass)					
	Picking					
Batch	1.57 ± 0.05 aA	1.19 ± 0.13 bB	0.76 ± 0.07 bC	0.53 ± 0.05 bD	0.36 ± 0.02 aD	1.05 ± 0.28 aB
Continuous	1.74 ± 0.09 aA	1.68 ± 0.16 aA	0.87 ± 0.06 aC	0.70 ± 0.09 aD	0.47 ± 0.09 aE	1.12 ± 0.05 aB
	Cutting					
Batch	1.66 ± 0.04 bA	1.53 ± 0.19 bA	0.87 ± 0.07 bB	0.84 ± 0.08 aB	0.46 ± 0.03 bC	1.67 ± 0.28 aA
Continuous	1.81 ± 0.07 aB	2.64 ± 0.21 aA	1.01 ± 0.06 aC	0.94 ± 0.08 aC	0.61 ± 0.09 aD	1.87 ± 0.07 aB
	Pulling					
Batch	1.72 ± 0.04 bA	1.60 ± 0.19 bA	0.91 ± 0.07 bB	0.92 ± 0.08 bB	0.51 ± 0.04 bC	1.80 ± 0.28 bA
Continuous	1.95 ± 0.10 aC	2.86 ± 0.17 aA	1.15 ± 0.07 aD	1.28 ± 0.10 aD	0.93 ± 0.17 aE	2.21 ± 0.11 aB

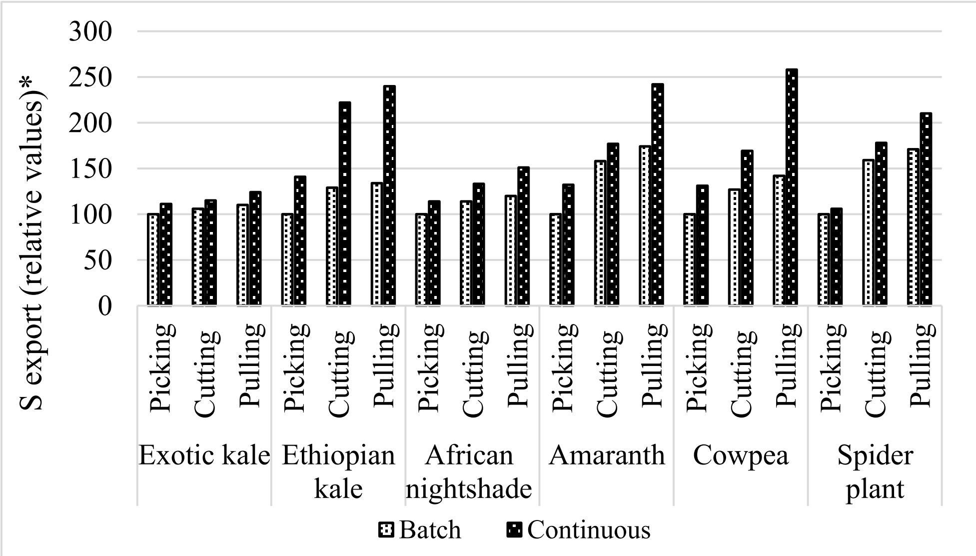

Figure 4.6: Effect of harvest technique (cutting, picking, pulling) and production system (batch, continuous) on S export from soil to market in different vegetable species; *for each species, the N export in the batch system harvested by picking was set to 100%.

4.4.4.5 Effects of production systems and harvesting techniques on Mg removal

The vegetable species removed different amount of Mg with respect to production system and harvesting technique (Table 4.18). The amount of Mg removed ranged from 0.28 kg per 10^3 kg leaf fresh mass for picking Ethiopian kale in the batch system to 1.65 kg per 10^3 kg leaf fresh mass for pulling amaranth in continuous system. Ethiopian kale and African nightshade always exported more Mg in the ontinuous production system than in the batch system, regardless of harvesting technique. Pulling the plants during harvest resulted in a higher Mg export in the continuous system than in the batch system for all species, with the exception of exotic kale. Among all vegetable species, across the production systems and harvesting techniques, amaranth removed much higher amounts of Mg. Ethiopian kale and African nightshade removed least amouonts of Mg in the batch production system.

Table 4.18: Yield-based magnesium removal (kg per 10^3 kg leaf fresh mass) with harvested plant organs from soil to market as affected by species, production system and harvest method (mean ± standard deviation). Means with the same lower case letters within the columns show no significant difference between the two production systems for each of the harvest methods analysed, means with the same upper case letters within rows indicate no significant differences among species ($p > 0.05$, ANOVA, LSD test, n = 4).

Production system	Species					
	Exotic kale	Ethiopian kale	African nightshade	Amaranth	Cowpea	Spider plant
	Mg removal (kg per 10^3 kg leaf fresh mass)					
	Picking					
Batch	0.41 ± 0.02 aC	0.28 ± 0.03 bD	0.32 ± 0.02 bD	0.94 ± 0.08 aA	0.55 ± 0.03 aB	0.43 ± 0.06 aC
Continuous	0.40 ± 0.03 aC	0.39 ± 0.02 aC	0.42 ± 0.03 aC	0.97 ± 0.05 aA	0.66 ± 0.11 aB	0.44 ± 0.02 aC
	Cutting					
Batch	0.44 ± 0.02 aC	0.35 ± 0.04 bD	0.41 ± 0.05 bCD	1.29 ± 0.09 aA	0.63 ± 0.04 bB	0.67 ± 0.04 aB
Continuous	0.43 ± 0.03 aE	0.58 ± 0.04 aD	0.51 ± 0.04 aDE	1.27 ± 0.05 aA	0.81 ± 0.12 aB	0.71 ± 0.04 aC
	Pulling					
Batch	0.46 ± 0.02 aC	0.37 ± 0.04 bD	0.43 ± 0.05 bCD	1.40 ± 0.10 bA	0.65 ± 0.05 bB	0.70 ± 0.04 bB
Continuous	0.49 ± 0.03 aC	0.63 ± 0.05 aC	0.56 ± 0.04 aC	1.65 ± 0.08 aA	0.95 ± 0.17 aB	0.87 ± 0.07 aB

Relative values showed, that Ethiopian kale exported more than twice the amount of Mg was exported, when harvested by cutting and pulling (Fig. 4.7). Cutting or pulling Ethiopian kale resulted in greater differences in Mg export between the two productions systems when compared to the other species. African nightshade and spider plant exported 1.5 times more Mg, when harvested by cutting or pulling in the continuous production system. This increase was also observed in amaranth and cowpea harvested by pulling in continuous system. In the batch production system, spider plant harvested by cutting or pulling removed more than 1.5 times of Mg. There were almost no effects of production system or harvest technique on Mg export visible in exotic kale.

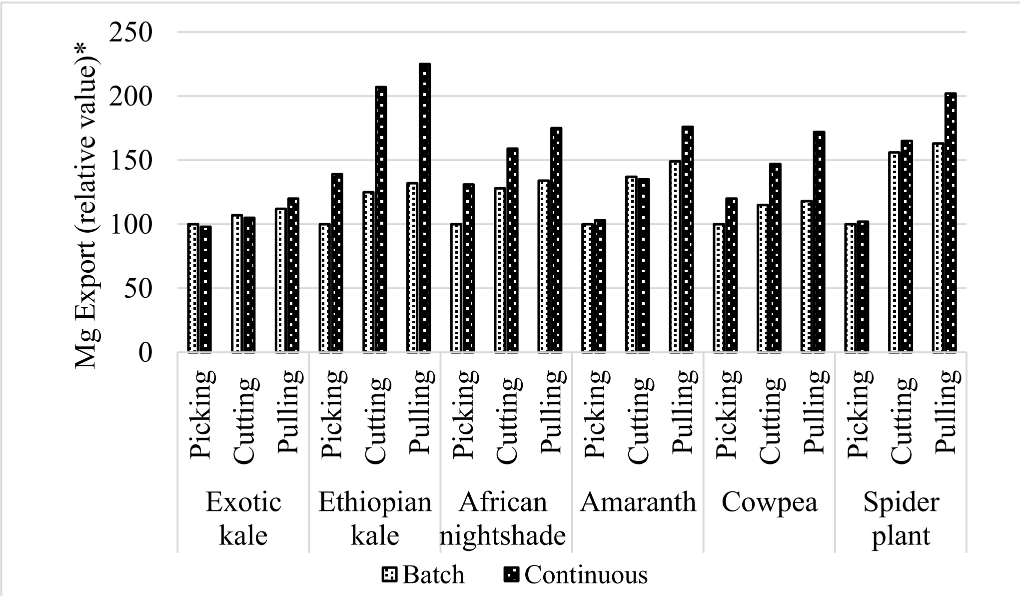

Figure 4.7: Effect of harvest technique (cutting, picking, pulling) and production system (batch, continuous) on Mg export from soil to market in different vegetable species; *for each species, the N export in the batch system harvested by picking was set to 100%.

4.4.4.6 Effects of production systems and harvesting techniques on Ca removal

Ca removal among vegetable species differed according to production system and harvesting method (Table 4.19) The range among species for Ca removal varied from 2.9 kg per 10^3 kg leaf fresh mass for African nightshade harvested by picking leaves in the batch production system to 9.1 kg per 10^3 kg leaf fresh mass for spider plant harvested by cutting or pulling in the batch system. When vegetables were harvested by picking, Ethiopian kale and cowpea exported significantly more Ca from the continuous system than from batch system. On the other hand, spider plant was the highest Ca exporter in the batch system, while cowpea exported least.

In the continuous system, picking of leaves resulted in similar Ca export in all species, apart from African nightshade. The behavior among species in Ca removal was different from other minerals. When harvested by pulling or cutting, species removed more Ca in the batch system. This was not observed for the previous described elements. There the highest amounts exported were found in the continuous system, when species showed significant differences. For instance, all species apart from cowpea removed a significantly higher amount of Ca in the batch system than in continuous system, when harvested by cutting. Pulling of vegetables during harvest removed higher amounts of Ca by exotic kale and African nightshade in the batch system and amaranth in the continuous system, when the production systems were compared. Among all species, spider plant and exotic kale grown under batch system removed more Ca from the soil. Ca export values for cutting and pulling did not differ for all species grown in the batch production system.

The relative values for exotic kale harvested by cutting or pulling showed a doubled export of Ca in the batch system (Fig. 4.8). All species that were grown in the batch system removed almost 1.5 times more of Ca when harvested by cutting or pulling. Ethiopian kale, African nightshade, amaranth, cowpea and spider plant exported at least 1.5 times more Ca when the continuous system was harvested by pulling. In spider plant, harvesting by picking the leaves resulted in a lower value of Ca removed.

Table 4.19: Yield-based calcium removal (kg per 10^3 kg leaf fresh mass) with harvested plant organs from soil to market as affected by species, production system and harvest method (mean ± standard deviation). Means with the same lower case letters within the columns show no significant difference between the two production systems for each of the harvest methods analysed, means with the same upper case letters within rows indicate no significant differences among species ($p > 0.05$, ANOVA, LSD test, n = 4).

Production system	Species					
	Exotic kale	Ethiopian kale	African nightshade	Amaranth	Cowpea	Spider plant
	Ca removal (kg per 10^3 kg leaf fresh mass)					
	Picking					
Batch	4.2 ± 0.4 aAB	3.4 ± 0.3 bCD	2.9 ± 0.1aD	4.1 ± 0.4 aBC	3.5 ± 0.3 bCD	4.9 ± 0.9 aA
Continuous	4.1 ± 0.1 aA	4.3 ± 0.5 aA	3.0 ± 0.2 aB	4.1 ± 0.2 aA	4.3 ± 0.5 aA	4.2 ± 0.1 aA
	Cutting					
Batch	8.3 ± 0.7 aA	6.6 ± 0.6 aB	5.6 ± 0.3 aB	6.6 ± 0.8 aB	5.4 ± 0.6 aB	9.1 ± 1.7 aA
Continuous	4.2 ± 0.2 bC	5.4 ± 0.6 bB	3.6 ± 0.2 bD	5.4 ± 0.3 bB	4.8 ± 0.5 aC	6.3 ± 0.2 bA
	Pulling					
Batch	8.3 ± 0.7 aA	6.7 ± 0.6 aB	5.6 ± 0.3 aB	6.6 ± 0.8 bB	5.4 ± 0.6 aB	9.1 ± 1.7 aA
Continuous	4.5 ± 0.2 bC	5.7 ± 0.6 aB	4.5 ± 0.3 bC	8.0 ± 0.5 aA	5.2 ± 0.6 aBC	7.4 ± 0.4 aA

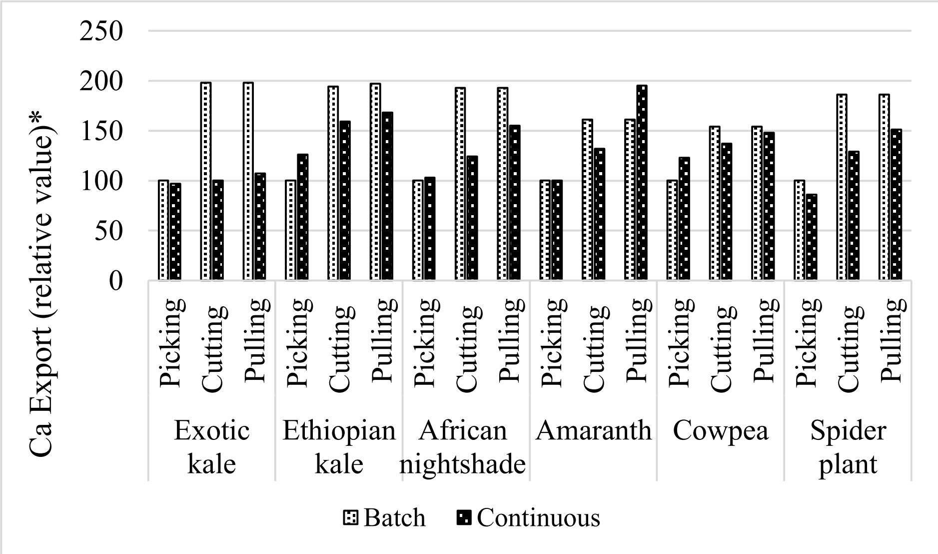

Figure 4.8: Effect of harvest technique (cutting, picking, pulling) and production system (batch, continuous) on Ca export from soil to market in different vegetable species; *for each species, the N export in the batch system harvested by picking was set to 100%.

4.4.5 Potential for saving nutrients in batch and continuous systems through optimization of harvest technique

In Kenya, most farmers harvest their vegetables by pulling. As seen in the results above, pulling carried lots of nutrients with the harvested organs from the soil to the market along with the non-edible organs, which are fed to animals or are used as fuel during vegetable preparation. In figures 4.9 and 4.10, the amount of nutrients was determined, that can be saved in both production systems, when vegetables are picked rather than pulled at harvest.

Considering batch production system, the results showed that harvesting exotic kale by picking leaves saved less than 20% of N, K, Mg and S, from being exported to the market whereas 22% and 49% of P and Ca are saved, respectively (Fig 4.9). In Ethiopian kale, between 24 – 26% of N, Mg and S was saved by picking leaves rather than pulling. Harvesting of Ethiopian kale also saved 37% P, 33% K and 49% Ca. African nightshade harvested by picking reduced exports of N, P, K and Mg between 25% and 32%, whereby 16% S and 48% Ca were the lowest and highest values of all saved minerals. Production and harvest of amaranth by picking saved about 35% N, 58% P, 59% K, 33% Mg, 42% S and 38% Ca, whereas in cowpea 9% N, 11% P, 19% K, 15 Mg, 29% S and 35% Ca were saved from export. More than 30% of all measured nutrients were saved when the spider plant was harvested by picking instead of pulling, saving 55 % K.

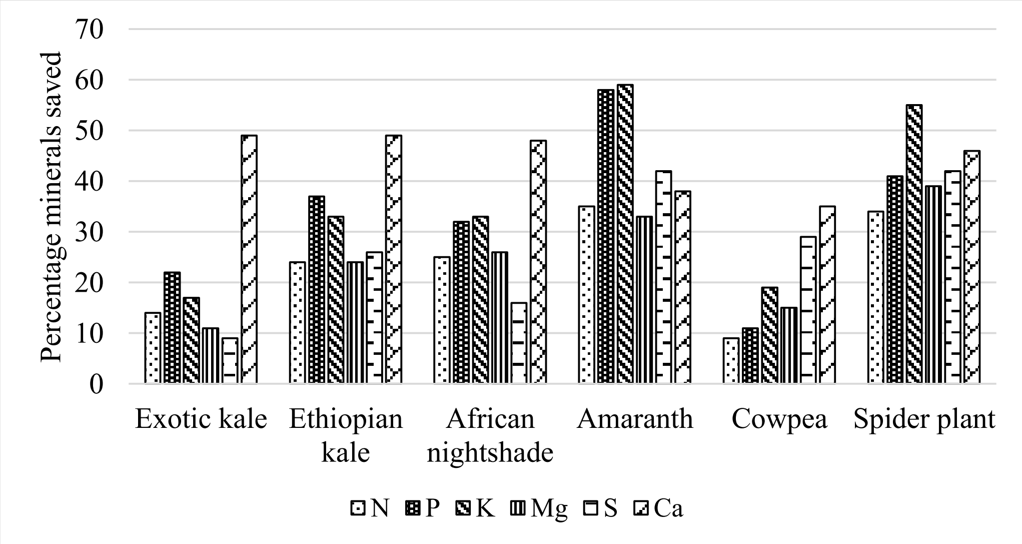

Figure 4.9: Amount of nutrients (percentage) saved on export to the market when harvest method changed from pulling to picking in the batch system. The nutrients removed (kg per 10^3 kg leaf fresh mass) in specific vegetable species through picking were compared to those removed by pulling and then made up to percentage (n = 4).

More nutrients were saved in the continuous system compared to the batch system, when vegetables were harvested by picking rather than pulling (Fig. 4.10). Exotic kale saved the least nutrients ranging from 6% for Ca to 23 % and 24 % for K and P respectively, thus different harvesting techniques of exotic kale does not have a big impact on nutrient export as opposed to other species. Picking of Ethiopian kale saved over 40% of N, P, K and S from export as well as 38% Mg and 25% Ca in the continuous system. For African nightshade, picking saved between 24% and 30% of N, Mg, S, Ca and over 40% of P and K. Amaranth could save a huge amount of nutrients when harvested by picking, saving up to 33% N, 65% P, 60% K, 41% Mg, 45% S and 49% Ca. On the other hand, cowpea saved 17% Ca and 49% S. Picking spider plant saved 35 % of N and more than 40% of all other minerals exports.

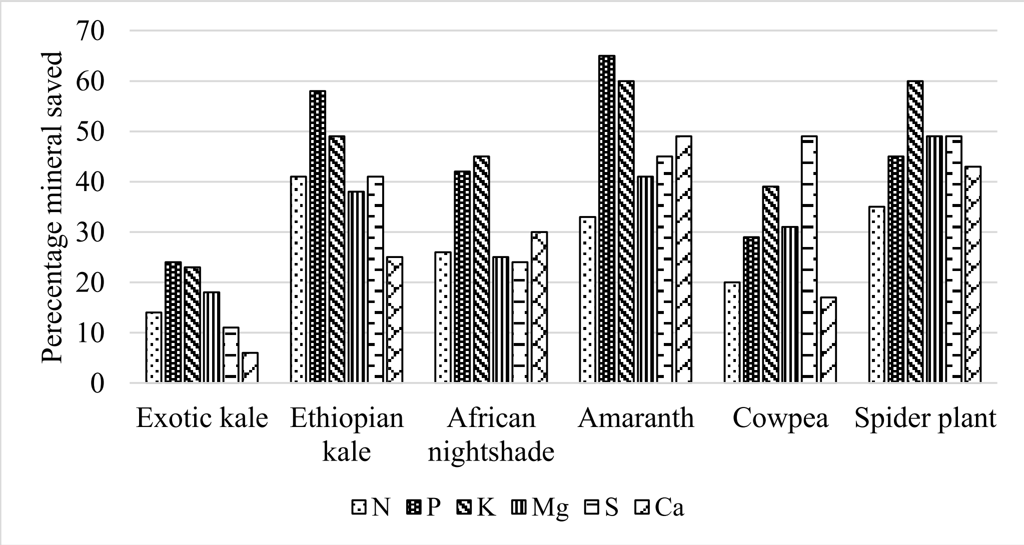

Figure 4.10: Amount (percentage) of nutrients saved from exportation to the market when vegetables in the continuous system are harvested by picking. The nutrients removed (kg per 10^3 kg leaf fresh mass) in specific vegetable species through picking were compared to those removed by pulling and then made up to percentage (n = 4).

4.5 Discussion

4.5.1 Effect of species, harvest technique and production system on nutrient fluxes from soil to market

The study showed that vegetable species differed in the amount of nutrients they export to the market from the soil. This can be attributed to physiological nutrient demand of the plant species or germplasm (Vanlauwe et al. 2015). Majumdar et al. (2016), outlined that plant genotype, root system and harvest index affected plant nutrient uptake and export. Overall, spider plant removed higher amounts of N and P, while kales exported a lot of S from the soil to the market (Table 4.14, 4.15 and 4.17, respectively). Nevertheless, amaranth exported high levels of K and Mg, which were approximately three times higher than the least exporters in the pulling system (table 4.15 and 4.18). This clearly showed that these vegetables differ in most of their physiological characteristics, which affected demand for particular nutrients.

Past study found out that species indeed differed in the amount of nutrients exported. Abdalla et al. (2012) found leafy vegetables to differ in nutrient export with cowpea having the highest export of N (39 kg N per 10^3 kg dry mass) followed by (in decreasing order) purslane (*Portulaca oleracea*), radish (*Raphanus raphanistrum subsp. sativus*), Jew's mallow

(*Corchorus olitorius*) and rocket (*Eruca vesicaria ssp. sativa*). The amounts of P and K exported were higher in cowpea and Jew's mallow, respectively. The nutrient exports for cowpea in this study by Abdalla et al. (2012) were similar to our study for N (assuming that dry mass was equivalent to 10% of fresh mass) and slightly lower for P (0.38 kg P per 10^3 fresh mass) and K (3.1 kg K per 10^3 fresh mass). Diogo et al. (2010) found amaranth to export more N (41 kg per 10^3 dry mass), P (6.3 kg per 10^3 dry mass) and K (64 kg per 10^3 dry mass) than lettuce - *Lactuca sativa*, 35 N kg, 5.9 kg P, 72 kg K per 10^3 dry mass), cabbage - *Brassica oleracea var. capitate* (33 kg N, 6.8 kg P, 36 kg K per 10^3 dry mass) and tomatoes - *Solanum lycopersicum* (27 kg N, kg per 10^3 dry mass). In findings of Diogo et al. (2010) it was clear, that leafy vegetables exported more nutrients from the soil compared to fruit vegetables. Other studies that have found variations of nutrient export include Lu et al. (2011) on tomatoes, radish (*Raphanus raphanistrum subsp. sativus*) and pakchoi (*Brassica rapa subsp. chinensis*), Siegfried et al. (2011) on cropping sequence involving carrot (*Daucus carota subsp. Sativus*), cauliflower (*Brassica oleracea var. botrytis*), and radish (*Raphanus raphanistrum subsp. sativus*), and Khai et al. (2007) on 20 different vegetable tin species in South Asia. It should be deduced from findings of Diogo et al. (2010) on nutrient export in millet (21 kg N, 5.2 kg P, 12 kg K per 10^3 dry mass), that vegetables are heavy exporters of minerals from the soil compared to cereals or pulses. Also Safi et al. (2011) found N, P, K export in three farming systems to be highest in vegetables compared to cereals and grapes, for instance with 46% of total N applied, followed closely by cereals (40%) and grapes (14%).

Plant biomass and mineral allocation among vegetable organs also played a major role in mineral export. For instance, the kales and African nightshade obtained the highest total yield compared to the remaining three species (Table 4.9) and thus are likely to export more nutrients from the soils compared to those species that produced low yield under the same nutrient application. Biomass allocation was highest towards leaves, followed by the stems in all the vegetable species. Given that high amounts of nutrients were found in the leaves, undoubtedly most nutrients are lost through harvesting of leaves and transport to the market. Our findings are in agreement with Liu et al. (2010), whose study revealed that nearly 55% of N exportss came from harvested crop organs (35%) and crop residues (20%). For our case, the harvested organs are the leaves, while the residues are the stems, stubbles and coarse roots. The differences in biomass allocation affected the nutrient export among species. At harvest, removal of plant organs led to both non – productive losses and losses, which are beneficial to human beings in form of nutrients. The beneficial losses comprised mainly of leaves (edible organs), while the non – productive were made up of stems, stubbles and coarse roots. Thus, differences in nutrient loss among species can also be derived from the plant organs that are transported to the market. In Kenya, most farmers harvest their vegetables by pulling and then transfer all the nutrients to the market without considering replenishment of lost nutrients to the soil. Considering all the nutrients measured, harvesting by picking transported less nutrients compared to cutting and pulling of the vegetables (Table 4.14 – 4.19). This was expected, because in cutting and pulling, non-edible plant parts which could be incorporated into soil after harvesting are also transported to the market. Considering nutrient export relative values, exotic kale did not show large differences in nutrient export among the harvesting techniques, especially for N, S and Mg. Ethiopian kale and amaranth, showed a big difference (more than 1.5 times) between picking in the batch system and pulling both production systems for most nutrients. Thus amaranth and Ethiopian kale should be preferably harvested by picking of leaves to avoid heavy export of nutrients.

Production systems did not differ in nutrient export in most species, when vegetables were harvested by picking, because at the time of harvest the leaves contained the maximum amount of nutrients needed and no translocation had occurred as a result of deficiency or for production of reproductive organs in the plant, in both production systems. When pulling or cutting, most vegetables showed the highest nutrient export in the continuous system, especially Ethiopian kale, African nightshade and cowpea. Some vegetables did not show any difference in nutrient export between the production systems. The differences were an indication that nutrient accumulation in the vegetable species, especially in the stems of the plant, varies with time and was species-specific. The longer the plant stays on the farm, the higher the amount of nutrients and carbon incorporated into biomass. Thus, for the soil organic matter enrichment and effective nutrient replenishment through non-edible plant organs, continuous method can be the most preferred system. As discussed before, pulling can deprive the soil more nutrients, especially in continuous production of vegetable species. A survey study by Onyuka et al. (personal communication) showed soils from AIVs growing areas in Kenya (Kisii and Kakamega County) to be very low in N, P and S. These could be attributed to vegetable species majorly grown in that parcel of land, harvesting techniques and production methods.

Although we were interested in measuring harvest related nutrient removal in our study, which included edible and non-edible organs, some of the nutrients may have been lost through leaching, soil erosion and, in case of N, by denitrification. Leaching of nutrients, especially N and K, can be difficult to extrapolate, which basically depends on nutrient form applied, soil moisture and rainfall pattern and amount. Since our experiment involved use of irrigation water, it could have leached the nutrients deep to the ground, where plant roots cannot reach. Predotova et al. (2011), found leaching in vegetable garden in Niger to remove 0.7 kg P ha^{-1} $year^{-1}$, while N varied according to soil texture. For our study, it was anticipated that most leaching occurred in the batch system, where vegetables were harvested and the ground left without cover. Since some minerals had not been fully utilized and were still in the soil, they could be leached away. Neeteson et al. (2003), did a study in field vegetable production and stated that large amounts of residual soil mineral N was lost through leaching after harvesting vegetables.

On the other hand, denitrification was possible. Our experiment involved the use of calcium ammonium nitrate as a source of N. There were possibilities of N losses as a result of ammonia volatilization, which highly depended on soil pH, texture and climatic factors. Our soils, that were finely tilled, provided room for exchange of oxygen due to aeration. Denitrification is likely to occur in the top 10 cm of fine textured soils (Neeteson and Carton 2001). There was speculation of lower denitrification in vegetables compared to cereals. When vegetables are grown under continuous system regeneration of new leaves is allowed after harvest of older leaves and this gives room for carbon build up throughout the roots, which reduces the chances of denitrification unlike in cereals, where harvesting is only done once. Also exposure to direct sunlight without soil cover activates the denitrification process, which could be higher in the batch system than continuous system after harvesting. It was found out, that after first and second round harvesting of AIVs in the continuous system, the vegetables covered over 50% of the ground, thus minimizing exposure and N loss.

Erosion also contributed to nutrient losses in our experiment, which, according to Stoorvogel and Smaling (1990), is attributed to soil crop/species cover. The review article reported soil loss in Kenya under maize to be 20 – 40 t ha^{-1} $year^{-1}$, 10 – 15 t ha^{-1} $year^{-1}$ for vegetables and

15 – 20 t ha^{-1} $year^{-1}$ for tea and coffee. The amount of nutrients removed through erosion depends on the topography of the area (slope gradient) and field management as well. Wind and water can move the soil from one point to another. In our experiment possible erosion could be minimal and in case of occurrence it was likely to be in the batch system, when the plants were harvested and ground left bare for some time, unlike in the continuous system, where the crops covered the soil for a longer period. Also, harvesting by pulling exposed the soil to erosion and thus results to nutrient loss and this should be avoided.

Our study could not quantify these losses and thus an extensive research has to be conducted to bridge this gap and come up with correct findings on vegetables and specifically AIVs. Loss of nutrients through leaching, denitrification and soil erosion are not as large as those from harvested organs and plant residues. In a study done in Kisii, Kakamega and Embu counties in Kenya, Van den Bosch et al. (1998), estimated the annual losses per hectare due to leaching at 53 kg N, due to gaseous emissions at 24 kg N and due to erosion at 28 kg N, 10 kg P and 33 kg K. These losses are site specific, depending on factors like topography, physical soil characteristics and weather.

4.5.2 Fertilizer demand of vegetable species relative to maize

The above discussion shows that vegetables export more nutrients and differ greatly in the type and amount of nutrients exported. The amount of nutrients exported from the farm with the products sold on the market is closely related to the yield levels. The differences between species in the total dry matter (Table 4.10 and 4.11) of our results reflect the site-specific species-environmental interaction, amaranth and spider plant were the most productive and cowpea the least productive. Under other environmental conditions (temperatures, occurrence of pests and diseases, availability of water), the ranking of species with regard to productivity would have been possibly different.

To obtain more generally applicable data on the amount of nutrients removed from the farm with the products and to derive species-specific fertilizer recommendations, we calculated the amount of nutrients removed from the farm per 10^{-3} kg of edible yield (leaves and tender laterals) for all species.

The nutrient concentrations of the edible plant organs in the batch system were used to calculate the nutrient ratios in the edible organs of vegetables compared to those of the edible organs (grains) of maize to compare the optimal fertilizer formulations for maize and leafy vegetables. The comparison was done with maize, as maize is the common food crop majorly grown in sSA and there were farmers who adopted the fertilizer requirements and recommendations for maize also for leafy vegetables. In addition, farmers prefered maize and fertilized more maize rather than vegetables.

Table 4.20: Nutrient ratios in edible organs of maize and vegetable species. Nutrient values for maize were taken from Hoskinson et al. 2007 and Johnson et al. 2010. Nutrient concentrations (kg per 10^3 kg edible yield) from vegetables species in our study were used to calculate nutrient ratios of leafy vegetables. The amount of N per 10^3 kg edible yield was set to 1.

Species	N	P	K	S	Mg	Ca
	Nutrient ratio in edible organs					
Maize	**1**	**0.30**	**0.31**	**0.16**	**0.11**	
Exotic kale	1	0.13	1.11	0.36	0.09	0.95
Ethiopian kale	1	0.16	1.45	0.34	0.08	0.97
African nightshade	1	0.18	1.53	0.25	0.11	0.96
Amaranth	1	0.22	1.72	0.13	0.22	0.98
Cowpea	1	0.15	1.13	0.09	0.14	0.90
Spider plant	1	0.22	0.83	0.16	0.07	0.75
ø leafy vegetable	**1**	**0.18**	**1.30**	**0.22**	**0.12**	**0.92**

The ratio of nutrients in vegetables differed among species (Table 4.20). Amaranth and spider plant had the highest P ratio among all the species, while the K ratio in amaranth was twice that of spider plant. This indicated that amaranth requires more P and K compared to other species and thus fertilizer amount of P and K should not be same as in other species. The amount of S in edible organs of exotic kale and Ethiopian kale was almost four times that of cowpea. This means that the fertilisation of S should be carried out separately for cales and cow peas, whereby the cales should be fertilised with four times more fertiliser than cow peas. For kale and the spider plant, the proportion of Mg was lower compared to the other three species and highest for amaranth. Ca ratio did not differ much in all vegetable species. A big difference between the average nutrient ratio of vegetables and that of maize was found. Out of one unit of N needed by maize, you need 0.30 units of P (about 2 times more than vegetables), 0.31 units of K (about 4 times less than vegetables). This clearly shows that the fertilizer formula for maize, especially for the main nutrients N, P and K, is unsuitable for the fertilization of leafy vegetables.

Considering the minimum duration maize takes on the farm (120 days) and comparing it with vegetables grown in this time period, it was found that maize exported much less nutrients through harvested organs when compared to vegetables (Fig. 4.11). It is estimated that in Kenya, farmers produce an average of 2 tons ha^{-1} of maize grain in 120 days and 60 tons of vegetables per ha^{-1} (3 seasons of vegetables grown in 120 days, AFDB 2015). From the data it was clear that more than 6 times N, more than 3 times P, more than 25 times K, more than 4 times S and more than 5 times Mg might be exported by vegetables compared to maize. Some species were bigger exporters than others, like spider plant, which exported 390 kg N ha^{-1} as opposed to cowpea that exported 180 kg N ha^{-1}. Highest and lowest nutrient exporters were: spider plant exported 85 kg P ha^{-1} and African nightshade exported 32 kg P ha^{-1}, amaranth exported 432 kg K ha^{-1} and cowpea 264 kg K ha^{-1}, exotic kale exported 94 kg S ha^{-1} and cowpea 22 kg S ha^{-1}, amaranth exported 56 kg Ca $ha^{-1}$and Ethiopian kale 17 kg Ca ha^{-1}. The higher export of nutrients from leafy vegetables requires that farms where vegetables are grown are fertilised with fertilisers with a suitable formula to maintain soil fertility. The choice of vegetables is of the utmost importance in order to prevent strong nutrient exporters being cultivated on less fertile soils or in the case of limited fertilizer availability.

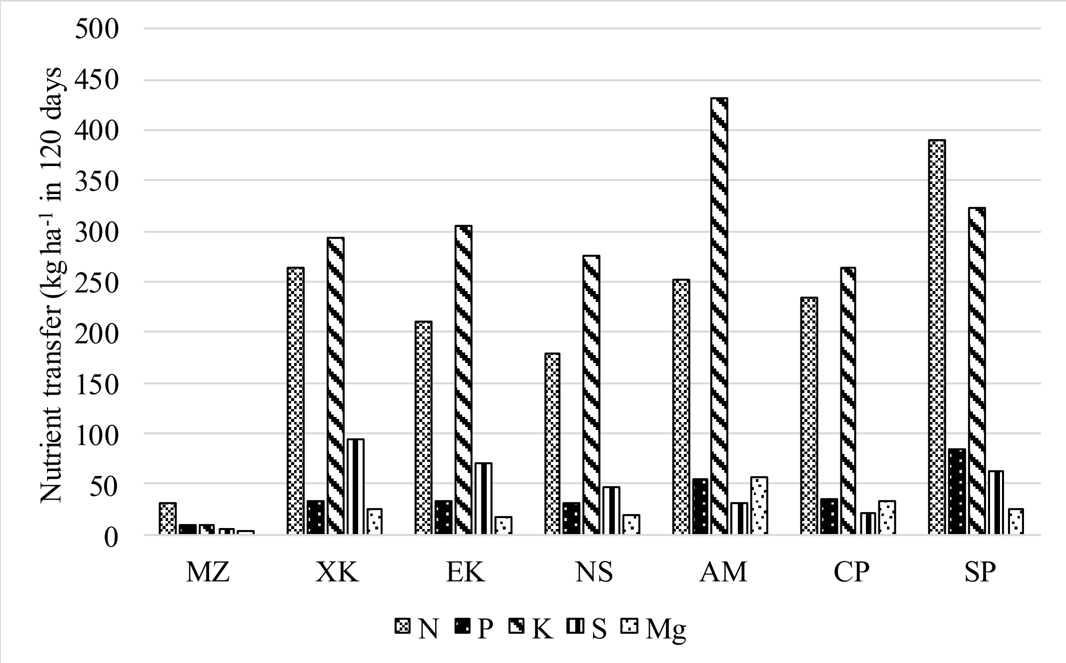

Figure 4.11: Nutrient export in maize and vegetable species in kg ha^{-1} within 120 days of growth. The average yield of maize was 2 tons per ha and of vegetables 60 tons per ha. Mineral concentrations (kg per kg 10^3 edible yield) of vegetables species from our study were used to calculate amounts of nutrients exported. Nutrient values for maize were taken from Hoskinson et al. 2007 and Johnson et al. 2010; MZ: maize, XK: exotic kale, EK: Ethiopian kale, NS: African nightshade, AM: amaranth, CP: cowpea, SP: spider plant.

4.6 Conclusion and recommendation

Leafy vegetables, specifically AIVs, can play a major role in soil nutrient balance. It was found, that these vegetables exported a lot of nutrients from the soil to the market compared to exotic kale and to some extend maize or cereals. Thus, for farmers to get a better yield they need to apply more fertilizer to these vegetables to avoid soil nutrient depletion. Farmers should be made aware that the recommended fertilizer formula for maize does not contain the optimal nutrient composition for vegetables and is therefore unsuitable for vegetable fertilization.

On the other hand, for leafy vegetables the amount of nutrients leaving the farm per 10^3 kg of edible yield strongly differs among species. Furthermore, the ratio of different nutrients (e.g. the ratio of N to S) strongly differs. Thus, the fertilizer recommendation for leafy vegetables should be species-specific with regard to total amount and nutrient ratio in fertilizer.

In the study, fewer nutrients were primarily lost when plant leaves were harvested, than pulling of whole plant, which also depended on the vegetable species. Thus, harvest related nutrient losses can be considerably reduced by harvesting only edible organs (picking) instead of pulling the whole plant. Farmers can control the export of nutrients by choosing the pickung harvesting technique and vegetable species.

The choice of production method also played a role in some vegetable species, especially on yield, where it was found that Ethiopian kale and African nightshade had a higher total yield in batch system, while exotic kale did best in continuous system. Furthermore, the continuous system resulted in higher levels of nutrient replenishment at the end of the growth cycle compared with the batch system and considering growth duration. Use of continuous system may lead to minimal amount of fertilizer application unlike for the batch system, where fertilizer should be applied every planting time, because the initially applied may have been lost through other means (leaching, erosion etc.).

The fertilization recommendation is based on the amount of individual nutrients that the plant needs for optimal plant growth and on the knowledge of the optimal/potential yield of the species. In order to provide the soil with sufficient nutrients, nutrients extracted from the soil by crops in the previous growing season, nutrient losses by leaching, erosion and gaseous emission as well as nutrients introduced into soil by irrigation, nitrogen fixation and atmospheric deposition should be taken into account. Besides selecting the appropriate plant species, the farmer can reduce the amount of nutrients extracted from the soil by by the choice of production system and harvest technology. Thus, putting all the above factors into consideration will lead to better fertilizer recommendation for achieving maximum productivity.

5.0 Summary

AIVs have recently captured considerable attention as "super vegetables" due to their nutritional and environmental benefits (Cernansky 2015). As AIVs include many species belonging to different botanical families, more species-specific knowledge e.g. on nutritional value and agronomic management is needed to fully exploit those benefits. In this thesis we compared leafy AIV species from five families including C_3 and C_4 species, and a legume and non-legume species (*Brassica carinata*/Cruciferae - Ethiopian kale, *Amaranthus cruentus*/Amaranthaceae - amaranth, *Vigna unguiculata*/Leguminosae cowpea, *Solanum scabrum*/Solanaceae - African nightshade, *Cleome gynandra*/Capparaceae - spider plant) with a non-indigenous species, which is commonly grown in Kenya (*Brassica oleracea acephala/*Cruciferae - "exotic kale"). We compared the leaf concentrations of beneficial and heavy metals as an index for the nutritional value, the performance under different rates and forms of phosphorus (P) supply as an index for adaptation to suboptimal chemical soil conditions, and the harvest-related nutrient outflow from soil as an index for fertilizer need. The thesis was imbedded in the interdisciplinary research program HORTINLEA (Horticultural Innovation and Learning for Improved Nutrition and Livelihood in East Africa).

For the assessment of leaf mineral element concentrations, we analysed 146 samples collected from open-air and supermarkets in Nairobi during the dry and wet season. The element concentrations significantly differed among species. For example, amaranth had particularly high concentrations of K, Mg, Ca, Fe and Zn. Spider plant was characterized by particularly high concentrations of P, Ca and Cu. Cowpea contained high Mn concentrations, while exotic kale showed higher concentrations of S and Ca. The effect of market type (open air vs. supermarket) on leaf mineral concentrations was negligible. Mineral concentrations in most vegetable species did not differ between seasons, apart from K, for which concentration in all species was higher in the wet than in the dry season. Exotic kale, Ethiopian kale and African nightshade had higher Fe concentration in the wet season.

Our data indicate that AIV may substantially contribute to the recommended dietary intake (RDI) of mineral elements for humans. For example a daily dish of 400 g vegetable fresh mass depending on the specific species would contribute for Fe from 34% to 321% of RDI, for Zn 16% to 51%, for Mg 81% to 284% and for Ca 58% to 168% of RDI. The potentially high contribution of AIV to the RDI of Fe should be critically questioned, however, as high leaf iron concentrations might have been the consequence of externally adhering ferrous dust/soil particles.

Just like the essential mineral concentrations also the leaf concentrations of the heavy metals Cd and Pb showed no differences between supermarkets and open air markets. In most AIVs, the leaf Cd concentrations were higher in the dry than in the wet season. African nightshade had somewhat higher Cd and Pb concentrations than other species. However, in nearly all samples from all species, the concentrations of Cd and Pb were within the permissible levels for leafy vegetables (WHO/FAO 2006).

Plant performance under different rates and forms of P supply was assessed in a pot experiment in which plant were supplied with 49 mg P in form of soluble KH_2PO_4, or phytate or poorly soluble rock P or $FePO_4$. In a control treatment no P was applied. Shoot fresh mass (i.e. agronomic yield) for all species was similar with P supply in form of KH_2PO_4 and phytate indicating that all species were effective in using organic P sources. However, species differed in their ability to use poorly soluble inorganic phosphates for yield production. Spider plant had very low yield when supplied with either rock P or FePO4. Amaranth and Ethiopian kale were particularly effective in using rock P, and Ethiopian kale and "exotic" kale were effective in using FePO4 for yield production. The low ability of spider plant to use poorly soluble inorganic P sources was associated with both, low uptake efficiency and low internal utilization efficiency. Species also differed in their root morphology responses to low P supply. For example, P deficiency was associated with a decrease in mean root diameter and an increase of specific root length in African nightshade, whereas specific root length of amaranth was decreased. In spider plant and amaranth, root hair density increased under conditions of P deficiency, whereas root hair density was not modified by P supply in the other species. Species also differed in their rhizosphere pH. In spider plant and both kales rhizosphere pH was above 6 when the plants were well supplied with P, and decreased to below 5 under conditions of P deficiency. In African nightshade, amaranth and cowpea, rhizosphere pH was below 4 when plants were well supplied with P in form of KH_2PO_4, and slightly increased under conditions of P deficiency. It is concluded that generalization about P efficiency of AIV species is not justified. To inform farmers about the best choice of AIV species under specific farm conditions, species-specific knowledge about P efficiency and site-specific knowledge about the most important P forms in soil is needed.

For the assessment of harvest-related nutrient outflow from soil, which is an index for fertilizer demand, we conducted a field experiment. At harvest we measured the nutrient accumulation separately in fine and coarse roots, stubble, stems and edible organs (laterals and leaves). We compared two cropping systems: In the "batch" system, the crop was completely harvested, clearing the field for the next crop on the same field. In the "continuous" system, at harvest one and two only the edible plant organs were harvested leaving the roots and basal 10 - 15 cm of the shoot in the field. At the third harvest plants were completely harvested. Furthermore, three harvesting methods were compared for the batch system and the last harvest of the continuous system. Plants were either uprooted, cut 5 cm above the soil surface, or only edible organs were harvested. To obtain general data on harvest-related nutrient fluxes, we calculated for all species the nutrient accumulation in various plant organs per ton of edible yield. The harvest-related nutrient outflow from soil was dependent on AIV species and harvest technique. In the "batch" system, harvest by pulling out the plant removed between 4 kg N per ton edible yield for nightshade and 10 kg N per ton edible yield for spider plant. The harvest-related P outflow per ton edible yield varied between 0.65 kg P for cowpea and 2.38 kg P for spider plant. The largest amounts of N, P and Ca were exported from soil with harvest of spider plant. The largest amounts of K and Mg were exported in amaranth, and the largest amounts of S were exported with African kale and "exotic" kale.

The harvest technique considerably influenced the amount of nutrients exported from soil to market. Harvesting only edible plant organs resulted in highest savings of exported nutrients compared to harvest by pulling the complete plant, as expected. For example, nutrient export by spider plant could be reduced by 35 % for N, 40 % for P and 56 % for Ca, by changing harvest method. In case of amaranth nearly 60 % of P and Mg could be saved. Change of the harvest technique to picking leaves reduced export of Ca by 35 % up to 50 % in all AIVs. The amounts of N, P, K and Mg saved form export in exotic kale and cowpea were lower, ranging between 10 % und 20 %.

The production system had no influence on yield of amaranth, cowpea and spider plant. Exotic kale had higher yield in the "batch" system whereas the yield of Ethiopian kale and nightshade was higher in the continuous system. When harvesting just edible parts, almost no effect of production system on nutrient export was observed. When harvested by cutting, spider plant and amaranth exported higher amounts of P, K and Ca in the “batch” system. Generally, export of calcium was higher in the “batch” system, except in cowpea. On the other hand, P, K, S and Mg were exported in higher amounts in the “continuous” system, especially when plants were pulled for harvest. High amounts of nutrients could be saved by optimizing harvest procedures in “continuous” system as well. To summarize, our data show that recommendations about the amount and composition of fertilizer input, which is necessary to replace harvest-related nutrient losses from soil should be species-specific, and should also take into consideration the harvest technique and the production system.

In conclusion, the data of this thesis showed that generalizations about AIV should be avoided. Significant differences among AIV species with regard to their nutritional value, their performance under suboptimal P supply and in fertilizer requirements make it very clear that each species should be evaluated separately.

5. Zusammenfassung

Durch ihre Umwelt- und Nährwertvorteile erweckten Afrikanische indigene Blattgemüse – „African Indigenous Vegetables“ (AIV) in letzter Zeit Aufmerksamkeit als „Supergemüse“ (Cernansky 2015). Da mehrere Spezies aus unterschiedlichen botanischen Familien innerhalb der AIVs vertreten sind, ist ein besseres Verständnis Spezies spezifischer Eigenschaften, wie z.B. Ernährungswert und agronomischem Management nötig, um diese Vorteile voll ausschöpfen zu können. In dieser Arbeit haben wir Afrikanisch indigene Blattgemüsearten aus fünf Familien, inklusive C_3 and C_4 Spezies und Leguminosen und nicht-Leguminosen (*Brassica carinata*/Cruciferae - Ethiopian Kale, *Amaranthus cruentus*/Amaranthaceae - Amaranth, *Vigna unguiculata*/Leguminosae - Cowpea, *Solanum scabrum*/Solanaceae - African Nightshade, *Cleome gynandra*/Capparaceae - Spiderplant) mit einer importierten und in Kenia häufig angebauten Gemüseart (*Brassica oleracea acephala/*Cruciferae - Exotic Kale) verglichen. Wir betrachteten die Pflanzenarten hinsichtlich ihrer Konzentrationen an wertgebenden und toxischen Elementen in Blättern als Index für den Nährwert, ihrer Reaktionen auf unterschiedliche zur Verfügung gestellten Mengen und Formen an Phosphor (P) als Index für die Adaptation an suboptimale chemische Bodenbedingungen und ihre erntebedingten Nährstoffausfuhren aus dem Boden als Index für den Düngerbedarf. Die Arbeit war in das interdisziplinäre Forschungsprogramm HORTINLEA (Horticultural Innovation and Learning for Improved Nutrition and Livelihood in East Africa) eingebunden.

Für die Beurteilung der Elementkonzentrationen in Blättern haben wir 146 Proben von Open Air- und Supermärkten in Nairobi während der Trocken- und Regenzeit gesammelt und untersucht. In den unterschiedlichen AIV Spezies gab es signifikante Unterschiede in den Nährstoffkonzentrationen. So wurden, zum Beispiel, in Amaranth hohe Konzentrationen an K, Mg, Ca, Fe und Zn gefunden. Spiderplant enthielt hohe Konzentrationen an P, Ca und Cu, Cowpea mehr Mn und Exotic Kale höhere Konzentrationen an S und Ca. Der Effekt des Markttyps auf die Nährstoffkonzentration war vernachlässigbar. Die Elementkonzentrationen in den meisten Gemüsearten zeigten keine Unterschiede zwischen der Regen- und Trockenzeit, abgesehen von Kalium, welches in höheren Konzentrationen während der Regenzeit in allen Spezies gefunden wurde. Exotic Kale, Ethiopian Kale und African Nightshade zeigten zudem auch höhere Konzentrationen an Eisen während der Regenzeit. Aus unseren Daten geht hervor, dass AIV Spezies wesentlich dazu beitragen können die empfohlenen täglichen Aufnahmemengen an Mineralstoffen für Menschen zur Verfügung zu stellen. Der Verzehr einer Gemüsemahlzeit von 400 g Frischgewicht könnte je nach Spezies z.B. die empfohlenen täglichen Aufnahmemengen an Eisen zu 34% bis 321% decken, an Zink zu 16% bis 51%, an Mg zu 81% bis 284% und an Ca von 58% bis zu 168%. Der Anteil an Eisen, welcher durch AIV aufgenommen werden kann, sollte kritisch betrachtet werden, da anhaftende, eisenhaltige Staub-/Bodenpartikel die Ursache für die höheren gemessenen Eisenkonzentrationen in den Blättern sein können.

Wie bei den gewünschten Nährstoffen zeigte sich auch bei den Schwermetallgehalten in den Gemüsen kein Effekt zwischen Open Air- und Supermarkt. In Bezug auf die Jahreszeiten wurden in den meisten AIVs höhere Cadmiumkonzentrationen während der Trockenzeit gefunden als in der Regenzeit, was auf die Bleigehalte nicht zutraf. Innerhalb der AIVs hatte African Nightshade die höchsten Konzentrationen an Cadmium und Blei. Dabei wurden die von der WHO/ FAO 2006 festgesetzten erlaubten Höchstkonzentrationen bei allen gemessenen Cadmium- und Bleikonzentrationen nicht überschritten.

Die Reaktionen der Pflanzen auf unterschiedliche Mengen und Formen an Phosphor wurden mit Hilfe eines Gefäßversuchs untersucht. Dabei wurden die Pflanzen jeweils mit 49 mg P versorgt, zum einen in löslicher P-Form mittels KH_2PO_4, zum andern mit organisch gebundenem P in Form von Phytat oder mit schwer löslichen Phosphat in Form von Rohphosphat, bzw. $FePO_4$. Zusätzlich gab es eine Variante in der kein P zugegeben wurde. Die Sprossfrischmasse (agronomischer Ertrag) war für alle Spezies ähnlich, wenn P in Form von KH_2PO_4 oder Phytat zur Verfügung stand, was darauf hinweist, dass alle Spezies die organische P-Quelle effektiv nutzen konnten. Die Spezies unterschieden sich jedoch in Ihrer Fähigkeit schwer lösliche P-Quellen zur Ertragsbildung zu nutzen. Spiderplant hatte sehr geringe Erträge mit Rohphosphat oder auch $FePO_4$ als P-Quelle. Amaranth und Ethiopian Kale hingegen konnten Rohphosphat sehr gut nutzen, Ethiopian Kale und Exotic Kale konnten $FePO_4$ effektiv zur Ertragsbildung nutzen. Die geringe Fähigkeit von Spiderplant die schwer löslichen P- Angebotsformen verwerten zu können stand in Zusammenhang mit geringer Aufnahmeeffizienz als auch geringer interner Nutzungseffizienz. Die Spezies unterschieden sich auch hinsichtlich ihrer Reaktionen der Wurzelmorphologie auf niedrige Phosphorverfügbarkeit. Unter P-Mangel kam es zur Abnahme des Wurzeldurchmessers und zur Zunahme der spezifischen Wurzellänge in African Nightshade, wohingegen die spezifische Wurzellänge in Amaranth abnahm. In Spiderplant und Amaranth erhöhte sich die Wurzelhaardichte unter P-Mangel, wohingegen sich die Wurzelhaardichte der anderen Spezies nicht änderte. Ebenso wurde der pH-Wert der Rhizosphäre innerhalb der unterschiedlichen Spezies unterschiedlich beeinflusst. Bei Spiderplant und beiden Kales lag der pH-Wert der Rhizosphäre unter optimaler Versorgung mit P bei über 6, sank aber auf Werte unter 5 unter P-Mangelbedingungen. Bei African Nightshade, Amaranth und Cowpea lag der pH-Wert der Rhizosphäre unter 4 bei Zugabe von KH_2PO_4 und stieg leicht an unter P-Mangelbedingungen. Daraus wurde geschlossen, dass keine generelle Aussage zur P-Effizienz der AIV Spezies getroffen werden kann. Um Bauern beraten zu können, welche Spezies zum Anbau auf einer bestimmten Farm ausgewählt werden sollte, benötigt man Kenntnis der Spezies spezifischen P-Effizienz und Kenntnis über die P-Formen im Boden der Farm.

Die Untersuchung der erntebedingten Nährstoffströme aus dem Boden, als Index für den Düngebedarf, wurde mittels eines Feldexperimentes bestimmt. Zur Ernte wurde die Menge an Nährstoffen in Fein- und Grobwurzeln, Stoppeln, Stängeln und verzehrbaren Organen (Blätter und Seitentriebe) gemessen. Wir verglichen zwei Anbausysteme: Im „Batch“ System wurden die gesamten Pflanzen geerntet und die nächsten Pflanzen wurden anschließend auf demselben Feld angebaut. Im „Continuous“ System wurden bei der ersten und zweiten Ernte nur die essbaren Pflanzenteile geerntet, wobei Wurzeln und die unteren 10-15 cm des Sprosses auf dem Feld verblieben. Bei der dritten Ernte wurde die komplette Pflanze geerntet. Zusätzlich wurden drei Erntemethoden für das „Batch“ System und die letzte Ernte des „Continuous“ Systems miteinander verglichen. Dabei erfolgte die Ernte entweder durch Herausziehen der gesamten Pflanze, Abschneiden der Pflanze 5 cm über dem Boden oder Abschneiden nur der essbaren Pflanzenteile. Zum Vergleich der erntebedingten Nährstoffexporte vom Feld wurden für alle Spezies die Nährstoffgehalte einzelner Pflanzenorgane pro Tonne essbarem Ertrag berechnet. Es wurde gezeigt, dass der Nährstoffaustrag aus dem Boden abhängig ist von den einzelnen Pflanzenarten, als auch der Erntetechnik. Betrachtet man das “Batch“ Produktionssystem in dem die Ernte durch Herausziehen der gesamten Pflanze erfolgte, so wurden im Fall von Stickstoff zwischen 4 kg N pro Tonne Blattfrischmasse bei Nightshade und 10 kg N pro Tonne Blattfrischmasse bei Spiderplant vom Feld ausgetragen. Bei Phosphor waren es zwischen 0,65 kg P pro Tonne

Blattfrischmasse (Cowpea) und 2,38 kg P pro Tonne Blattfrischmasse (Spiderplant), die ausgetragen wurden. Insgesamt wurden die höchsten Mengen an Stickstoff, Phosphor und Calcium durch Spiderplant ausgetragen. Der Austrag von Kalium und Magnesium erfolgte hauptsächlich durch Amaranth. Die beiden Kalearten exportierten besonders viel Schwefel. Die Höhe der Nährstoffexporte wurde in Abhängigkeit von betrachteter Pflanzenart und Nährstoffe durch Anwendung der beiden anderen Erntetechniken reduziert. Die Ernte nur der essbaren Planzenteile führte erwartungsgemäß zu den deutlichsten Einsparungen im Vergleich zum Herausziehen der gesamten Pflanze. So wurde der Nährstoffentzug von, z.B. Spiderplant durch Ernte nur der essbaren Teile um 35 % bei Stickstoff, 40% bei Phosphor und 56 % bei Calcium gesenkt. Bei Amaranth konnten knapp 60% an Phosphor und Magnesium eingespart werden. Der Calciumaustrag wurde bei allen Spezies um 35 % bis 50 % gesenkt. Geringer fielen die eingesparten Mengen an exportiertem Stickstoff, Phosphor, Kalium und Magnesium bei Exotic Kale und Cowpea aus, sie bewegten sich in einem Bereich zwischen 10 % und 20 %.

Der Vergleich der Produktionssysteme zeigte keinen Einfluss auf die Erträge von Amaranth, Cowpea und Spiderplant. Exotic Kale lieferte höhere Erträge im "Batch" System, Ethiopian Kale und Nightshade im "Continuous" System. Erntet man ausschließlich die Blätter, so sind die Nährstoffausträge in beiden Produktionssystemen ähnlich. Schneidet man die Pflanzen zur Ernte ab führt dies bei Spiderplant und Amaranth für Phosphor, Kalium und Calcium zu höheren Austrägen im "Batch" System. Für Calcium fand sich generell ein höherer Austrag im "Batch" System, mit Ausnahme von Cowpea. Phosphor, Kalium, Schwefel und Magnesium hingegen wurden tendenziell vermehrt im "Continuous" System entzogen, v.a. beim Ernten durch Herausziehen der Pflanze. Auch im "Continuous" System können große Mengen an Nährstoffen durch eine optimierte Erntemethode eingespart werden. Letztendlich zeigen unsere Daten, dass Empfehlungen zur Menge und Zusammensetzung von einzusetzendem Dünger, welcher die erntebedingten Nährstoffverluste aus dem Boden ersetzen soll, abgestimmt sein sollte auf die angebaute Spezies, dem Produktionssystem und der Erntetechnik.

Die Daten dieser Arbeit zeigen, dass verallgemeinerte Aussagen zu den AIVs vermieden werden sollten. Es wurden signifikante Unterschiede der einzelnen AIVs hinsichtlich ihres Nährwerts, ihres Umgangs mit suboptimaler Phosphorversorgung und im Düngerbedarf gefunden, so dass für eine genaue Bewertung jeder aufgeführte Gesichtspunkt für jede Gemüseart separat betrachtet werden sollte.

6.0 References

Abdalla, S. B., Predotova, M., Gebauer, J. and Buerkert, A. (2012): Horizontal nutrient flows and balances in irrigated urban gardens of Khartoum, Sudan. Nutrient Cycling in Agroecosystems, 92(2), 119–132. http://doi.org/10.1007/s10705-011-9476-7.

Abdu, N., Agbenin, J. O. and Buerkert, A. (2011): Phytoavailability, human risk assessment and transfer characteristics of cadmium and zinc contamination from urban gardens in Kano, Nigeria. Journal of the Science of Food and Agriculture, 91(15), 2722–2730. http://doi.org/10.1002/jsfa.4583.

Abel, S. (2011): Phosphate sensing in root development. Current Opinion in Plant Biology, 14(3), 303–309. http://doi.org/10.1016/j.pbi.2011.04.007.

Abugre, C., Appiah, F. and Kumah, P. (2011): The effect of time of harvest and drying method on the nutritional composition of spider flower (Cleome gynandra L). International Journal of Postharvest Technology and Innovation, 2(3), 221. http://doi.org/10.1504/IJPTI.2011.043327.

Abukutsa Onyango, M. O. (2010): Strategic repositioning of African indigenous vegetables in the Horticultural sector.

Adam A. (2016): Der Nährstoff- und Mineralstoffgehalt von vier ausgewählten afrikanischen indigenen Blattgemüsearten (Amaranthus cruentus, Cleome gynandra, Solanum scabrum und Vigna unguiculata) - eine Literaturrecherche.

AFDB (2015): Cereal Crops: Rice, Maize, Millet, Sorghum, and Wheat. Background paper

Ahenkora, K., Adu Dapaah, H. K. and Agyemang, A. (1998): Selected nutritional components and sensory attributes of cowpea (Vigna unguiculata [L.] Walp) leaves. Plant Foods for Human Nutrition, 52(3), 221–229. http://doi.org/10.1023/A:1008019113245.

Akombi, B. J., Agho, K. E., Merom, D., Renzaho, A. M. and Hall, J. J. (2017): Child malnutrition in sub-Saharan Africa: A meta-analysis of demographic and health surveys (2006-2016). PLoS ONE, 12(5), 1–11. http://doi.org/10.1371/journal.pone.0177338.

Ali, M. H. H. and Al-Qahtani, K. M. (2012): Assessment of some heavy metals in vegetables, cereals and fruits in Saudi Arabian markets. Egyptian Journal of Aquatic Research, 38(1), 31–37. http://doi.org/10.1016/j.ejar.2012.08.002.

Alkama, N. E., Bolou, B. B., Vailhe, H., Roger, L., Ounane, J. M. and Drevon, J .J. (2008): Genotypic variability in P use efficiency for symbiotic nitrogen fixation is associated with variation in proton efflux in cowpea rhizosphere. Soil Biol. Biochem. 41 (9), 1823–1824.

Allen, L., de Benoist, B., Dary, O. and Hurrell R. (eds) (2006): Guidelines on food fortification with micronutrients. World Health Organization, Geneva Also available via http://www.who.int/nutrition/publications/micronutrients/.

Ancuceanau, R., Dinu, M., Hovane, M. V., Anghel, A. I., Popescu, C. V. and Negre, S. (2015): A Survey of Plant Iron Content — A Semi-Systematic Review, 10320–10351. http://doi.org/10.3390/nu7125535.

Andal, F. A (2016): Assessment of the Possible Utilization of Tomato as a Phytoremediant in Soils Artificially Contaminated with Heavy Metals. International Journal of Applied Environmental Sciences ISSN 0973-6077 Volume 11, Number 1 (2016), pp. 193-209.

Angel, S. J., Baker, M. J. A., Whiting S. N. and Chaney, L. R. (2003): Soil moisture effects on uptake of metals by Thlaspi, Alyssum, and Berkheya. Plant and soil, 256: 325–332.

Ansah, G S., Adu-Dapaah, H., Kumaga, F., Gracen, V. and Nartey, F. K. (2016): Evaluation of cowpea (Vigna unguiculata L. Walp.) genotypes for phosphorus use efficiency. Acta Horticulturae, (1127), 373–380. http://doi.org/10.17660/ActaHortic.2016.1127.58.

ASDSP. (2015): Nairobi County. Agricultural Sector Development Support Programme. Retrieved from http://www.asdsp.co.ke/index.php/nairobi-county.

Bai, Z., Ma, L., Ma, W., Qin, W., Velthof, G. L., Oenema, O. and Zhang, F. (2016): Changes in phosphorus use and losses in the food chain of China during 1950–2010 and forecasts for 2030. Nutrient Cycling in Agroecosystems, 104(3), 361–372. http://doi.org/10.1007/s10705-015-9737-y.

Bain, L. E., Awah, P. K., Geraldine, N., Kindong, N. P., Sigal, Y., Bernard, N. and Tanjeko, A. T. (2013): Malnutrition in Sub - Saharan Africa: Burden, causes and prospects. Pan African Medical Journal, 15, 1–9. http://doi.org/10.11604/pamj.2013.15.120.2535.

Balemi, T. and Negisho, K. (2012): Management of soil phosphorus and plant adaptation mechanisms to phosphorus stress for sustainable crop production: A review. Journal of Soil Science and Plant Nutrition, 12(3), 547–561. http://doi.org/10.4067/S0718-95162012005000015.

Beegle, D. B. and Durst, P. T. (2002): Managing Phosphorus for Crop Production. Penn state extension. Agronomy Facts 13.

Bieleski, R. L. (1973): Phosphate pools, phosphate transport, and phosphate availability. Annu. Rev. Plant Physiol. 24, 225–252.

Birchfield S. (2008) Rootfly; software for Minirhizotron Image Analysis. https://cecas.clemson.edu/~stb/rootfly/Rootfly_help.html.

Birnin-Yauri, A. U., Yahaya, Y., Bagudo, U. B. and Noma, S. S (2011): Seasonal variation in nutrient content of some selected vegetables from Wamakko, Sokoto State, Nigeria. journal of Soil Science and Environmental Management Vol. 2(4), pp. 117-125.

Bittner, M. (2017): Effect of harvest technique and production system on yield, root architecture and carbon input into soil of Amaranth.

Brown, L. K., George T. S., Dupuy L. X. and White P. J. (2013): A conceptual model of root hair ideo types for future agricultural environments: what combination of traits should be targeted to cope with limited P availability? Ann Bot 112:317–330.

Brown, S. L., Chaney, R. L., Angle, J. S. and Baker, A. M. (1995): Zinc and cadmium uptake by hyperaccumulator Thlaspi caerulescens grown in nutrient solution. Soil Sci Am J 59: 125-133.

Bryla, D. R. and Duniway, J. M. (1997): Growth, phosphorus uptake, and water relations of safflower and wheat infected with an arbuscular mycorrhizal fungus. New Phytol. (1997), 136, 581-590.

Caulfield, E. L., Richard, A. S., Rivera, A. J., Musgrove, P. and Black E. R (2006). Stunting, Wasting, and Micronutrient Deficiency Disorders, Chapter 28; 551 – 567.

Cercamondi, C. I., Egli, I. M., Ahouandjinou, E., Dossa, R., Zeder, C., Salami, L. and Zimmermann, M. B. (2010): Afebrile Plasmodium falciparum parasitemia decreases absorption of fortification iron but does not affect systemic iron utilization : a double stable-isotope study in young Beninese women 1 – 3, (2). http://doi.org/10.3945/ajcn.2010.30051.Iron.

Cernansky, R., (2015): The rise of Africa's super vegetables: Long overlooked in parts of Africa, indigenous greens are now capturing attention for their nutritional and environmental benefits. Nature 522 (7555), 146-148

Chauvin, N. D., Mulangu, F. and Porto, G. (2012): Food Production and Consumption Trends in Sub-Saharan Africa: Prospects for the Transformation of the Agricultural Sector. Working Papers, 76. http://doi.org/10.1016/j.foodpol.2013.10.006

Clemens, S., Aarts M. G. M., Thomine S. and Verbruggen N. (2012). Plant science: the key to preventing slow cadmium poisoning. Vol. 18, issue 2; 92-99.

Chweya, J. A., Eyzaguirre, P. B. (eds). (1999): The biodiversity of traditional leafy vegetables. IPGRI.

Crusak, M. A. (1994): Iron Transport to Developing Ovules of Pisum safivud. Seed lmport Characteristics and Phloem Iron-loading Capacity of Source Regions. Plant Physiol. 104, 649-655.

Cunnigham, J. H., Milligan, G. and Trevisan L. (2001): Minerals in Australian fruits and vegetables- a comparison of levels between the 1980s and 2000, Food Standards, Australia and New Zealand, 10–16.

Dávila, O. G., Gómez-bernal, J. M. and Ruíz-huerta, E. A. (2011): Plants and Soil Contamination with Heavy Metals in Agricultural Areas.

De Graaff, J., Kessler, A. and Nibbering, J. W. (2011): Agriculture and food security in selected countries in Sub-Saharan Africa: Diversity in trends and opportunities. Food Security, 3(2), 195–213. http://doi.org/10.1007/s12571-011-0125-4.

Dickinson, N., Rankin, J., Pollard, M., Maleta, K., Robertson, C. and Hursthouse, A. (2014): Science of the Total Environment Evaluating environmental and social influences on iron and zinc status of pregnant subsistence farmers in two geographically contrasting regions of Southern Malawi. Science of the Total Environment, 500–501, 199–210. http://doi.org/10.1016/j.scitotenv.2014.08.087.

Diogo, R. V. C., Buerkert, A. and Schlecht, E. (2010): Horizontal nutrient fluxes and food safety in urban and peri-urban vegetable and millet cultivation of Niamey, Niger. Nutrient Cycling in Agroecosystems, 87(1), 81–102. http://doi.org/10.1007/s10705-009-9315-2.

Drechsel, P. (2002): Comparing soil nutrient depletion in typical urban, peri-urban and rural farming systems in Ghana. Practices and Constraints of Land and Water Resources Management in Urban and Peri-Urban Agriculture, 7.

Ekesa, B. N., Walingo, M. K. and Abukutsa-Onyango, M. O. (2009): Accesibility to and Consumption of Indigenous Vegetables and Fruits By Rural Households in Matungu Division, Western Kenya. African Journal of Food Agriculture Nutrition and Development, 9(8).

Faerge, J. and Magid, J. (2004): Evaluating NUTMON nutrient balancing in Sub-Saharan Africa. Nut. Cyc. Agroecosyst., 69, 101–110.

Fang, Y., Nie, Z., Liu, F., Die, Q., He, J. and Huang, Q. (2014): Concentration and health risk evaluation of heavy metals in market-sold vegetables and fishes based on questionnaires in Beijing, China. Environmental Science and Pollution Research, 21(19), 11401–11408. http://doi.org/10.1007/s11356-014-3127-x.

Fanzo, J. (2012): The Nutrition Challenge in Sub-Saharan Africa. UNDP - Regional Bureau for Africa - Working PAper, (January), 1–68.

FAO (2004a). Vitamin and mineral requirements in human nutrition: report of a joint FAO/WHO expert consultation, Bangkok, Thailand, 21–30 September 1998.

FAO (2004b): Scalling Soil Nutrient Balances. Enabling Mesolevel Application of African Realities. FAO Fertilizer and Plant Nutrition Bulletin (Vol. 53). http://doi.org/10.1017/CBO9781107415324.004.

FAO, & WHO (2001): Human Vitamin and Mineral Requirements. Human Vitamin and Mineral Requirements, 303. http://doi.org/10.1016/B978-0-323-06619-8.10013-1.

FAO (2005). United Nations Food and Agriculture Organization. Increasing fruit and vegetable consumption becomes a global priority. Available from: http://fao.org/english/newsroom/focus/2003/fruitveg1.htm.

Fontes, F. L. R., Pereira, J. M. N. and Neves, L. C. J. (2014): Uptake and translocation of Cd and Zn in two lettuce cultivars. http://dx.doi.org/10.1590/0001-37652014117912.

Frossard, E., Bucher, M., Ma, F., Mozafar, A. and Hurrell, R. (2000): Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. Journal of the Science of Food and Agriculture, 80, 861–879.

Gahoonia, T. S. and Nielsen, N. E. (1996): Variation in acquisition of soil phosphorus among wheat and barley genotypes. Plant and Soil, 223–230. http://doi.org/10.1007/BF00011587.

Gall, J. E. and Rajakaruna, N. (2013): The physiology, functional genomics, and applied ecology of heavy metal-tolerant Brassicaceae. Brassicaceae: Characterization, Functional Genomics and Health Benefits, 121–148.

Ganry, J. (2009): Current Status of Fruits and Vegetables Production and Consumption in Francophone African Countries - Potential Impact on Health. Human Health Effects of Fruits and Vegetables, 249–256.

George, T. S., Hinsinger, P. and Turner, B. L. (2016): Phosphorus in soils and plants – facing phosphorus scarcity, 1–6. http://doi.org/10.1007/s11104-016-2846-9.

Gibson, R. S., Wawer, A. A., Fairweather-tait, S. J., Hurst, R., Young, S. D., Broadley, M. R., Chilimba, C. D. A., Louise Ander, E., Watts J. M., Kalimbira A., Bailey B. K. and Siyame, E. W. P. (2015): Dietary iron intakes based on food composition data may underestimate the contribution of potentially exchangeable contaminant iron from soil. Journal of Food Composition and Analysis, 40, 19–23. http://doi.org/10.1016/j.jfca.2014.11.016.

Gonçalves, A., Goufo, P., Barros, A., Domínguez-Perles, R., Trindade, H., Rosa, E. A. S. and Rodrigues, M. (2016): Cowpea (Vigna unguiculata L. Walp), a renewed multipurpose crop for a more sustainable agri-food system: Nutritional advantages and constraints. Journal of the Science of Food and Agriculture, 96(9), 2941–2951. http://doi.org/10.1002/jsfa.7644.

Greenwood, D. J., Stellacci A. M., Meacham M. C., Broadley M. R. and White P. J. (2005): Components of P response of different Brassica oleracea genotypes are reproducible in different environments. Crop Science 45, 1728–1735.

Greffeuille, V., Kayodé, A. P. P., Icard-vernière, C., Gnimadi, M., Rochette, I. and Mouquet-rivier, C. (2011): Changes in iron, zinc and chelating agents during traditional African processing of maize : Effect of iron contamination on bioaccessibility, 126, 1800–1807. http://doi.org/10.1016/j.foodchem.2010.12.087.

Gruhn, P., Goletti, F. and Yudelman, M. (2000): Integrated Nutrient Management, Soil Fertility, and Sustainable Agriculture: Current Issues and Future Challenges. Food Policy. http://doi.org/ISBN 0-89629-638-5.

Grusak, M. A. (1994): Iron transport to developing ovules of Pisum sativum. I. Seed import characteristics and phloem iron-loading capacity of source regions. Plant Physiol. 104, 649-655.

Gunawardena, J., Egodawatta, P., Ayoko, G. A. and Goonetilleke, A. (2013): Atmospheric deposition as a source of heavy metals in urban storm water. Atmospheric Environment, 68, 235–242. http://doi.org/DOI 10.1016/j.atmosenv.2012.11.06.2

Gupta, S., Gowri, B. S. and Lakshmi, A. J. (2013): Retention of nutrients in green leafy vegetables on dehydration, 50(October), 918–925. http://doi.org/10.1007/s13197-011-0407-z.

Habwe, F. O. and Walingo, K. M. (2008): Food processing and preparation technologies for sustainable utilization of Africa indigenous vegetables for nutrition security and wealth creation in Kenya. Using Food Science and Technology to Improve Nutrition and Promote National Development.

Haileslassie, A., Priess, J., Veldkamp, E., Teketay, D. and Lesschen, J. P. (2005): Assessment of soil nutrient depletion and its spatial variability on smallholders' mixed farming systems in Ethiopia using partial versus full nutrient balances. Agriculture, Ecosystems and Environment, 108(1), 1–16. http://doi.org/10.1016/j.agee.

Hammond, J. P., Broadley, M. R., White, P. J., King, G. J., Bowen, H. C., Hayden, R. and Greenwood, D. J. (2009): Shoot yield drives phosphorus use efficiency in Brassica oleracea and correlates with root architecture traits. Journal of Experimental Botany, 60(7), 1953–1968. http://doi.org/10.1093/jxb/erp083.

Hart, A. D., Ajubuike, C. U., Barimalaa, I. S. and Achinewhu, S. C. (2005): Vegetable consumption pattern of households in selected areas of the old Rivers State of Nigeria. African J. of Food Agriculture Nutrition and Development Online 2005; 5(1). Available from http://www.ajfand.net/index.html Accessed October 19th 2005.

HCD (2016). Horticulture validated report 2014, 1 – 68.

Hengl, T., Leenaars, J. G. B., Shepherd, K. D., Walsh, M. G., Heuvelink, G. B. M., Mamo, T. and Kwabena, N. A. (2017): Soil nutrient maps of Sub-Saharan Africa: assessment of soil nutrient content at 250 m spatial resolution using machine learning. Nutrient Cycling in Agroecosystems, 109(1), 77–102. http://doi.org/10.1007/s10705-017-9870-x.

Hinsinger, P. (2001): Bioavailibility of soil inorganic P in the rhizosphere as effected by root-induced chemical changes. Plant and Soil, 237, 173–195. http://doi.org/10.1023/A:1013351617532.

Hinsinger, P., Plassard C., Tang C. and Jaillard B. (2003): Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review.

Hoeppner, N. (2017): Nutzung anorganischer und organischer Phosphorquellen durch verschiedene afrikanische Gemüsearten.

Hooda, P. S., Henry, C. J. K., Seyoum, T. A., Armstrong, L. D. M. and Fowler, M. B. (2004): The potential impact of soil ingestion on human mineral metabolism', Science of the Total Environment, vol 333, pp75–87.

Hoskinson, R. L., Karlen, D. L., Birrell, S. J., Radtke, C. W. and Wilhelm, W. W. (2007). Engineering, nutrient removal, and feedstock conversion evaluations of four corn stover harvest scenarios. Biomass Bioenergy 31, 126–136.

Icard-Vernière, C., Picq, C., Courbis, L. and Mouquet-Rivier C. (2016). The Type of Fortificant and the Leaf Matrix Both Influence Iron and Zinc Bioaccessibility in Iron-fortified Green Leafy Vegetable Sauces from Burkina Faso. The Royal Society of Chemistry, 1-3.

Imungi, J. K. and Potter, N. N. (1983): Nutrient Contents of Raw and Cooked Cowpea Leaves. Journal of Food Science, 48(4), 1252–1254. http://doi.org/10.1111/j.1365-2621.1983.tb09204.x.

Ingwersen, J. and Streck, T. (2005): A regional scale study on the crop uptake of cadmium from sandy soils: measurement and modeling. J. Environ. Qual. 34, 1026–1035.

IPNI (1999): Functions of Phosphorus in Plants. Better Crops, 83(1), 6–7.

Ismail, G. and Suffla S. (2013). Child Safety, Peace and Health Promotion Child Malnutrition.

JICA (2014): The Project on Integrated Urban Development Master Plan for the City of Nairobi in the Republic of Kenya Final Report (Draft), (May), 221. Retrieved from http://citymasterplan.nairobi.go.ke/index.php/downloads/doc_download/132-draft-final-report-main1.

Jiménez-Aguilar, D. M. and Grusak, M. A. (2015): Evaluation of Minerals, Phytochemical Compounds and Antioxidant Activity of Mexican, Central American, and African Green Leafy Vegetables. Plant Foods for Human Nutrition, 70(4), 357–364. http://doi.org/10.1007/s11130-015-0512-7.

Johnson, J. M. F., Wilhelm W. W., Karlen D. L., Archer D. W., Wienhold B., Lightle D. T. et al. (2010): Nutrient removal as a function of corn stover cutting height and cob harvest. BioEnergy Research, 3, 342–352.

Joy, E. J. M., Ander, E. L., Young, S. D., Black, C. R., Watts, M. J., Chilimba, A. D. C. and Broadley, M. R. (2014): Dietary mineral supplies in Africa. Physiologia Plantarum, 151(3), 208–229. http://doi.org/10.1111/ppl.12144.

Joy, E. J. M., Broadley, M. R., Young, S. D., Black, C. R., Chilimba, A. D. C., Ander, E. L. and Watts, M. J. (2015): Science of the Total Environment Soil type influences crop mineral composition in Malawi. Science of the Total Environment, The, 505, 587–595. http://doi.org/10.1016/j.scitotenv.2014.10.038.

Kabata-Pendias, A. and Pendias, H. (1989): Trace elements in the Soil and Plants. CRC Press, Boca raton, FL.

Kamga, R. T., Kouamé, C., Atangana, A. R., Chagomoka, T. and Ndango, R. (2013): Nutritional Evaluation of Five African Indigenous Vegetables. Journal of Horticultural Research, 21(1), 99–106. http://doi.org/10.2478/johr-2013-0014.

Kananke, T., Wansapala, J. and Gunaratne, A. (2014): Heavy Metal Contamination in Green Leafy Vegetables Collected from Selected Market Sites of Piliyandala Area, Colombo District, Sri Lanka. American Journal of Food Science and Technology, 2(5), 139–144. http://doi.org/10.12691/ajfst-2-5-1.

Kapungwe, E. M. (2013): Heavy Metal Contaminated Water, Soils and Crops in Peri Urban Wastewater Irrigation Farming in Mufulira and Kafue Towns in Zambia. Journal of Geography and Geology, 5(2), 55–72. http://doi.org/10.5539/jgg.v5n2p55.

Kawai, K., Saathoff, E., Antelman, G., Msamanga, G. and Fawzi, W. W. (2009): Geophagy (Soil-eating) in Relation to Anemia and Helminth Infection among HIV – Infected Pregnant Women in Tanzania, 80(1), 36–43.

Khai, N. M., Ha, P. Q. and Öborn, I. (2007): Nutrient flows in small-scale peri-urban vegetable farming systems in Southeast Asia-A case study in Hanoi. Agriculture, Ecosystems and Environment, 122(2), 192–202. http://doi.org/10.1016/j.agee.2007.01.003.

Kiba, D. I., Zongo, N. A., Lompo, F., Jansa, J., Compaore, E., Sedogo, P. M. and Frossard, E. (2012): The diversity of fertilization practices affects soil and crop quality in urban vegetable sites of Burkina Faso. European Journal of Agronomy, 38(1), 12–21. http://doi.org/10.1016/j.eja.2011.11.012.

Klug, B., Kirchner, T. W. and Horst, W. J. (2015). Differences in aluminium accumulation and resistance between genotypes of the genus Fagopyrum. Agronomy, 5, 418–434. http://dx.doi.org/10.3390/agronomy5030418.

KNBS. (2010): The 2009 Kenya Population and Housing Census - Population Distribution by Age, Sex and Administrative Units, IC, 546. Retrieved from http://www.knbs.or.ke/index.php?option=com_phocadownload&view=category&download=584:volume-1c-population-distribution-by-age-sex-and-administrative-units&id=109:population-and-housing-census-2009&Itemid=599.

Kruger, J., Mongwaketse, T., Faber, M., van der Hoeven, M., and Smuts, C. M. (2015): Potential contribution of African green leafy vegetables and maize porridge composite meals to iron and zinc nutrition. Nutrition, 31(9), 1117–1123. http://doi.org/10.1016/j.nut.2015.04.010.

Kruger, M., Sayed, N., Langenhoven, M. and Holing, F. (1998): Composition of South African Foods: Vegetables and Fruit. Medical Research Council's tables (MRC), 1988.

Kuchenbuch, R., Claassen, N. & Jungk, A. P (1986): Potassium availability in relation to soil moisture. Plant Soil (1986) 95: 233. Https://doi.org/10.1007/BF02375075.

Kumssa, D. B., Joy, E. J. M., Ander, E. L., Watts, M. J., Young, S. D., Rosanoff, A., White, P. J., Walker, S., and Broadley, M. R. (2015a): Global magnesium supply in the food chain. Crop & Pasture Science, 66, 1278–1289 http://dx.doi.org/10.1071/CP15096.

Kumssa, D. B., Joy, E. J. M., Ander, E. L., Watts, M. J., Young, S. D., Walker, S. and Broadley, M. R. (2015b): Dietary calcium and zinc deficiency risks are decreasing but remain prevalent. Nature Publishing Group, 1–11. http://doi.org/10.1038/srep10974.

Lambers, H., Martinoia, E. and Renton, M. (2015): Plant adaptations to severely phosphorus-impoverished soils. Current Opinion in Plant Biology, 25, 23–31. http://doi.org/10.1016/j.pbi.2015.04.002.

Lambers, H., Shane, M. W., Cramer, M. D., Pearse, S. J. and Veneklaas, E. J. (2006): Root structure and functioning for efficient acquisition of phosphorus: Matching morphological and physiological traits. Annals of Botany, 98(4), 693–713. http://doi.org/10.1093/aob/mcl114.

Lasat, M. M. (1997): The Use of Plants for the Removal of Toxic Metals from Contaminated Soil. American Association for the Advancement of Science Environmental Science and Engineering Fellow, 18, 359–362. http://doi.org/10.1080/09593331808616549.

Leon, J. and Schwang, K. U. (1992): Description and application of a screening method to determine root morphology traits of cereals cultivars. Z. Acker. Pflanzenbau 169, 128–134.

Li, H., Huang, G., Meng, Q., Ma, L. and Yuan, L. (2011): Integrated soil and plant phosphorus management for crop and environment in China. A review, 1, 157–167. http://doi.org/10.1007/s11104-011-0909-5.

Li, j., Xie, M. Z., Xu, M. J. and Sun, F. Y. (2006): Risk assessment for safety of soils and vegetables around a lead/zinc mine. Volume 28, Issue 1–2, pp 37–44.

Liang, L. Z., Shen, R. F., Yi, X. Y., Zhao, X. Q., Chen, Z. C., Chen, R. F. and Dong, X. Y. (2009): The phosphorus requirement of Amaranthus mangostanus L. exceeds the "change point" of P loss. Soil Use and Management, 25(2), 152–158. http://doi.org/10.1111/j.1475-2743.2009.00211.x.

Liao, M., Hocking, P. J., Dong, B., Delhaize, E., Richardson, A. E. and Ryan, P. R. (2008): Genotypic variation in phosphorus efficiency among wheat genotypes grown on two contrasting Australian soils. Aust J Agr Res 59:157–166.

Lim, S. S., Vos, T., Flaxman, A. D., Danaei, G., Shibuya, K., Adair-Rohani, H. and Ezzati, M. (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: A systematic analysis for the Global Burden of Disease Study. The Lancet, 380(9859), 2224–2260. http://doi.org/10.1016/S0140-6736 (12) 61766-8.

Liu, J., You, L., Amini, M., Obersteiner, M., Herrero, M., Zehnder, A. J. B. and Yang, H. (2010): A high-resolution assessment on global nitrogen flows in cropland. Proceedings of the National Academy of Sciences, 107(17), 8035–8040. http://doi.org/10.1073/pnas.0913658107.

Lu, H. J., Ye, Z. Q., Zhang, X. L., Lin, X. Y. and Ni, W. Z. (2011): Growth and yield responses of crops and macronutrient balance influenced by commercial organic manure used as a partial substitute for chemical fertilizers in an intensive vegetable cropping system. Physics and Chemistry of the Earth, 36(9–11), 387–394. http://doi.org/10.1016/j.pce.2010.03.030.

Lyu, Y., Tang, H., Li, H., Zhang, F., Rengel, Z., Whalley, W. R. and Shen, J. (2016): Major Crop Species Show Differential Balance between Root Morphological and Physiological Responses to Variable Phosphorus Supply. Frontiers in Plant Science, 7 (December), 1–15. http://doi.org/10.3389/fpls.2016.01939.

Ma, Z., Bielenberg, D. G., Brown, K. M. and Lynch, J. P. (2001): Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. Plant, Cell and Environment, 24(4), 459–467. http://doi.org/10.1046/j.1365-3040.2001.00695.x.

MacDonald, G. K., Bennett, E. M., Potter, P. A. and Ramankutty, N. (2011): Agronomic phosphorus imbalances across the world's croplands. Proceedings of the National Academy of Sciences, 108(7), 3086–3091. http://doi.org/10.1073/pnas.1010808108.

Mahan, L.K. and Escott-Stump, S. (2000). Krause's Food Nutrition and Diet Therapy (10th ed.). Philadelphia, Pa.: W.B. Saunders Company.

Majumdar, K., Sanyal, S. K., Kumar Dutta, S. K., Satyanarayana, T. and Singh, V. K. (2016): Nutrient Mining: Addressing the Challenges to Soil Resources and Food Security, 1–490. http://doi.org/10.1007/978-81-322-2716-8.

Makokha, A. O. and Ombwara F. (2005): Potential for increased use of indigenous Kenyan Vegetables as functional foods. In: In: Proceedings of the third Horticulture Workshop on Sustainable Horticultural Production in the Tropics, 26th -29th November 2003. Maseno University MSU, Maseno, Kenya. ISBN: 9966-758-11-9. pp 102-116.

Manara, A. (2012): Plants and Heavy Metals. Signal Transduction, 27–54. http://doi.org/10.1007/978-94-007-4441-7.

Manske, G. G. B., Ortiz-Monasterio, J. I., Van Ginkel, M., Gonzalez, R. M., Rajaram, S., Molina, E and Vlek P. L. G. (2000): Traits associated with improved P-uptake efficiency in CIMMYT's semi dwarf spring bread wheat grown on acid Andisols in Mexico. Plant Soil 221:189–204.

Marschner, H. (1993): Zinc uptake from soils. In: Robson AD (ed) Zinc in Soils and Plants. Kluwer, Dordrecht, the Netherlands, pp 59–77.

Marschner P. (2012). Marschner's Mineral nutrition of higher plants, 3rd edition Academic Press., London, UK:

Marschner, H. (1995): Marschner's Mineral nutrition of higher plants. 2nd edition Academic Press., London, UK.

Matti P. (1995): Image manipulation Program Features. https://www.gimp.org/about/prehistory.html

McGrath, S.P., Chang, A.C., Page, A.L. and Wilter, E. (1994): Land application of sewage sludge: scientific perspectives of heavy metal loading limits in Europe and the United States. Environ. Rev. 2, 108–118.

McLaughlin, M. J., Parker, D. R. and Clarke, J. M. (1999): Metals and micronutrients - Food safety issues. Field Crops Research, 60(1–2), 143–163. http://doi.org/10.1016/S0378-4290 (98)00137-3.

McLaughlin, M.J. and Singh, B.R. (2000): Cadmium in soil and plants: a global perspective. Dordrecht, The Netherlands: Kluwer Academic Publish 13-21.

Micheni, A. N., Kanampiu, F. K. and Rodriguez, D. (2011): Characterization of Soil Nutrient Levels in Smallholder Farms in Eastern Kenya. 5th WCCA & 3rd FSD, 4–6.

Miller, C. R., Ochoa, I., Nielsen, K. L., Beck, D. and Lynch, J. P. (2003): Genetic variation for adventitious rooting in response to low phosphorus availability: potential utility for phosphorus acquisition from stratified soils. Functional Plant Biology 30: 973–985.

Mithen, R., Bennett, R. and Marquez, J. (2010): Phytochemistry Glucosinolate biochemical diversity and innovation in the Brassicales. Phytochemistry, 71(17–18), 2074–2086. http://doi.org/10.1016/j.phytochem.2010.09.017.

Mohamed, M. A., Siddig, E. E., Elaagip, A. H., Edris, A. M. and Nasr, A. A. (2016): Parasitic contamination of fresh vegetables sold at central markets in Khartoum state, Sudan. 15:17. doi: 10.1186/s12941-016-0133-5.

Muchuweti, M., Birkett, J. W., Chinyanga, E., Zvauya, R., Scrimshaw, M. D. and Lester, J. N. (2006): Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: Implications for human health. Agriculture, Ecosystems and Environment, 112(1), 41–48. http://doi.org/10.1016/j.agee.2005.04.028.

Muhanji, G., Roothaert, R. L., Webo, C. and Stanley, M. (2011): African indigenous vegetable enterprises and market access for small-scale farmers in East Africa. International Journal of Agricultural Sustainability, 9(November), 194–202. http://doi.org/10.3763/ijas.2010.0561.

Mutune, N., Makobe, M. and Abukutsa-Onyango, M. O. O. (2013): Impact analysis of lead, copper and zinc content in selected African indigenous and exotic vegetables from Nairobi markets, Kenya. African Journal of Environmental Science and Technology, 7(6), 540–545. http://doi.org/10.5897/AJEST2013.1511.

Nabulo, G., Black, C. R. and Young, S. D. (2011): Trace metal uptake by tropical vegetables grown on soil amended with urban sewage sludge. Environmental Pollution, 159(2), 368–376. http://doi.org/10.1016/j.envpol.2010.11.007.

Nabulo, G., Origa, H. O., Nasinyama, G. W. and Cole, D. (2008): Assessment of Zn, Cu, Pb and Ni contamination in wetland soils and plants in the Lake Victoria basin. Int J. Environ. Sci. Tech, 5(1), 65–74. http://doi.org/10.1007/BF03325998.

Neeteson, J. J., Langeveld, J. W. A., Smit, A. L. and de Haan, J. J. (2003): Nutrient balances in field vegetable production systems. Toward Ecologically Sound Fertilization Strategies for Field Vegetable Production, (627), 13–23.

Neeteson, J. and Carton, O. (2001): The environmental impact of nitrogen in field vegetable production. Acta Horticulturae, 563(1), 21–28.

Neumann, G. and Martinoia, E. (2002): Cluster roots – an underground adaptation for survival in extreme environments. Trends in Plant Science, 7: 162–167.

Newman, A. L. and Reynolds, M. C. (2005): Bacteria and phytoremediation: new uses for endophytic bacteria in plants, VoL. 23, Issue 1; 6-8.

Ngugi, I., Gitau, R. and Nyoro, J. (2007). Access to high value markets by smallholder farmers of African Indigenous vegetables. Tegemeo Institute of Agricultural Policy and Development, Egerton University.

Niu, Y. F., Chai, R. S., Jin, G. L., Wang, H., Tang, C. X. and Zhang, Y. S. (2013): Responses of root architecture development to low phosphorus availability: A review. Annals of Botany, 112(2), 391–408. http://doi.org/10.1093/aob/mcs285.

Nriagu, J. O. (1979): Global inventory of natural and anthropogenic emissions of trace metals to the atmosphere. Nature 279: 1247-1294.

Nyenje, P. M., Foppen, J. W., Uhlenbrook, S., Kulabako, R. and Muwanga, A. (2010): Eutrophication and nutrient release in urban areas of sub-Saharan Africa - A review. Science of the Total Environment, 408(3), 447–455. http://doi.org/10.1016/j.scitotenv.2009.10.020.

Odhav, B., Beekrum, S., Akula, U. and Baijnath, H. (2007): Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa. Journal of Food Composition and Analysis, 20(5), 430–435. http://doi.org/10.1016/j.jfca.2006.04.015.

Oenema, O., Kros, H. and De Vries, W. (2003): Approaches and uncertainties in nutrient budgets: Implications for nutrient management and environmental policies. European Journal of Agronomy, 20(1–2), 3–16. http://doi.org/10.1016/S1161-0301 (03)00067-4.

Okoronkwo, N. E., Igwe, J. C. and Onwuchekwa, E. C. (2005): Risk and health implications of polluted soils for crop production. African Journal of Biotechnology, 4(13), 1521–1524.

Oladeji, O. O., Kolawole, G. O., Adeoye, G. O. and Tian, G. (2006): Effects of plant residue quality, application rate, and placement method on phosphorus availability from Sokoto rock phosphate. Nutrient Cycling in Agroecosystems, 76(1), 1–10. http://doi.org/10.1007/s10705-006-9021-2.

Omondi, E. O., Engels, C., Nambafu, G., Schreiner, M., Neugart, S., Abukutsa-onyango, M. and Winkelmann, T. (2017): Nutritional compound analysis and morphological characterization of spider plant (Cleome gynandra) - an African indigenous leafy vegetable. Food Research International, 100(April), 284–295. http://doi.org/10.1016/j.foodres.2017.06.050.

Omotayo, O. E. and Chukwuka, K. S. (2009): Soil fertility restoration techniques in sub-Saharan Africa using organic resources. African Journal of Agricultural Research, 4(3), 144–150.

Oniang 'o, R., Grum, M. and Obel-Lawson E. (2005): Developing African leafy vegetables for improved nutrition. African Journal of Food, Agriculture, Nutrition and Development (Vol. 7).

Onyuka, E., Nambafu, G., Holst, S., Bessler, H., Andika, D., Gweyi-Onyango, P. J., Mwonga, S. and Engels, C. (nd). Challenges for Soil fertility on AIV farms in Kenya: Socioeconomic and /or natural factors.

Othman, O. C. (2001): Heavy metals in green vegetables and soils from vegetable gardens in Dar es Salaam, Tanzania. Tanzania Journal of Science. http://doi.org/10.4314/tjs.v27i1.18334.

Palm, C. A., Gachengo, C. N., Delve, R. J., Cadisch, G. and Giller, K. E. (2001): Organic inputs for soil fertility management in tropical agro ecosystems: Application of an organic resource database. Agriculture, Ecosystems and Environment, 83, 27–42.

Pant, B. D., Burgos, A., Pant, P., Cuadros-Inostroza, A., Willmitzer, L. and Scheible, W. R. (2015): The transcription factor PHR1 regulates lipid remodeling and triacylglycerol accumulation in Arabidopsis thaliana during phosphorus starvation. Journal of Experimental Botany, 66(7), 1907–1918. http://doi.org/10.1093/jxb/eru535.

Pearse, S. J. (2011): Why does the musketeer approach to phosphorus acquisition from sparingly soluble forms fail: All for one, but not one for all? Plant and Soil, 348(1–2), 81–83. http://doi.org/10.1007/s11104-011-0975-8.

Pearse, S. J., Veneklaas, E. J., Cawthray, G., Bolland, M. D. A. and Lambers, H. (2006): Triticum aestivum shows a greater biomass response to a supply of aluminium phosphate than Lupinus albus, despite releasing fewer carboxylates into the rhizosphere. New Phytologist, 169(3), 515–524. http://doi.org/10.1111/j.1469-8137.2005.01614.x.

Pearse, S. J., Veneklaas, E. J., Cawthray, G., Bolland, M. D. A. and Lambers, H. (2007): Carboxylate composition of root exudates does not relate consistently to a crop species' ability to use phosphorus from aluminium, iron or calcium phosphate sources. New Phytologist, 173(1), 181–190. http://doi.org/10.1111/j.1469-8137.2006.01897.x.

Péret, B., Clément, M., Nussaume, L. and Desnos, T. (2011): Root developmental adaptation to phosphate starvation: Better safe than sorry. Trends in Plant Science, 16(8), 442–450. http://doi.org/10.1016/j.tplants.2011.05.006.

Perveen, S., Ihsanullah, I., Shah, Z., Nazif, W., Shah, S. S. and Shah, H. (2011): Study on accumulation of heavy metals in vegetables receiving sewage water. Journal of the Chemical Society of Pakistan, 33, 21-28.

Piggott J. J., Lange K., Townsend R. C. and Mathaei D. C. (2012): Multiple Stressors in Agricultural Streams: A Mesocosm Study of Interactions among Raised Water Temperature, Sediment Addition and Nutrient Enrichment. https://doi.org/10.1371/journal.pone.0049873.

Plaxton, W. C. and Tran, H. T. (2011): Metabolic Adaptations of Phosphate-Starved Plants. Plant Physiology, 156(3), 1006–1015. http://doi.org/10.1104/pp.111.175281.

Predotova, M., Bischoff, W. A. and Buerkert, A. (2011): Mineral-nitrogen and phosphorus leaching from vegetable gardens in Niamey, Niger. Journal of Plant Nutrition and Soil Science, 174(1), 47–55. http://doi.org/10.1002/jpln.200900255.

Raboy V. (2001): Seeds for a better future: 'low phytate' grains help to overcome malnutrition and reduce pollution. Volume 6, issue 10, p458-462, DOI: http://dx.doi.org/10.1016/S1360-1385(01)02104-5.

Radwan, M. A. and Salama, A. K. (2006): Market basket survey for some heavy metals in Egyptian fruits and vegetables. Food and Chemical Toxicology, 44(8), 1273–1278. http://doi.org/10.1016/j.fct.2006.02.004.

Raghothama, K. G. (1999): Phosphate acquisition. Annual Review of Plant Physiology 50, 665–693.

Ramaekers, L., Remans, R., Rao, I. M. and Blair, M. W. (2010): Strategies for improving phosphorus acquisition efficiency of crop plants, 1–26.

Rangnekar, S. S., Sahu, S. K., Pandit, G. G. and Gaikwad V.B. (2013: Study of Uptake of Pb and Cd by Three Nutritionally Important Indian Vegetables Grown in Artificially Contaminated Soils of Mumbai, India. Int. Res. J. Environment Sci., Vol. 2(9), 53-59.

Reddy, D. D., Rao, A. S. and Singh M. (2005): Changes in P fractions and sorption in an Alfisol following crop residues application. J. Plant Nutr. Soil Sci. (168), 241 – 247. DOI:10.1002/jpln.200421444.

Richardson, A. E., Lynch, J. P., Ryan, P. R., Delhaize, E., Smith, F. A., Smith, S. E. and Simpson, R. J. (2011): Plant and microbial strategies to improve the phosphorus efficiency of agriculture. Plant and Soil, 349(1–2), 121–156. http://doi.org/10.1007/s11104-011-0950-4.

Rieuwerts, J. S., Thornton, I., Farago, M. E., Ashmore, M. R., (1998): Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical load approach for metals. Chemical Speciation and Bioavailability 10, 61-75.

Rose, T. J., Hardiputra, B. and Rengel, Z. (2010): Wheat, canola and grain legume access to soil phosphorus fractions differs in soils with contrasting phosphorus dynamics. Plant and Soil, 326(1), 159–170. http://doi.org/10.1007/s11104-009-9990-4.

Rose, T. J., Liu, L. and Wissuwa, M. (2013): Improving phosphorus efficiency in cereal crops: Is breeding for reduced grain phosphorus concentration part of the solution? Frontiers in Plant Science, 4(November), 444. http://doi.org/10.3389/fpls.2013.00444.

Rose, T. J., Mori, A., Julia, C. C. and Wissuwa, M. (2016): Screening for internal phosphorus utilisation efficiency: comparison of genotypes at equal shoot P content is critical. Plant and Soil, 401(1–2), 79–91. http://doi.org/10.1007/s11104-015-2565-7.

Rose, T. J., Rose, M. T., Pariasca-Tanaka, J., Heuer, S. and Wissuwa, M. (2011): The Frustration with Utilization: Why Have Improvements in Internal Phosphorus Utilization Efficiency in Crops Remained so Elusive? Frontiers in Plant Science, 2(November), 1–5. http://doi.org/10.3389/fpls.2011.00073.

Rouached, H., Arpat, A. B. and Poirier, Y. (2010): Regulation of phosphate starvation responses in plants: Signaling players and cross-talks. Molecular Plant, 3(2), 288–299. http://doi.org/10.1093/mp/ssp120.

RSA (2015): Report of a Market Study on Fresh Vegetables Market in Kenya Consumer's Survey By Research Solutions Africa (RSA) Ltd, 1–37.

Ruel, M. T., Minot, N. and Smith, L. (2005): Patterns and determinants of fruit and vegetable consumption in sub-saharan Africa: a multicountry comparison. Joint FAO/WHO Workshop on Fruit and Vegetables for Health, 45.

Safi, Z., Predotova, M., Schlecht, E. and Buerkert, A. (2011): Horizontal matter fluxes and leaching losses in urban and peri-urban agriculture of Kabul, Afghanistan. Journal of Plant Nutrition and Soil Science, 174(6), 942–951. http://doi.org/10.1002/jpln.201000385.

Sanchez, P. A., Shepherd, K. D., Soule, M. J., Place, F. M., Mokwunye, A. U., Buresch, R. J., Kwesiga, F. R., Izac, A-M. N., Ndiritu, C. G. and Woomer, P. L. (1997): Soil fertility replenishment in Africa: An investment in natural resource capital. pp 1-46. In (R.J. Buresch, P.A. Sanchez and F. Calhoon, Eds.) Replenishing Soil Fertility in Africa. Soil Science Society of America Special Publication No. 51. Madison, Wisconsin.

Sardar K., Ali S., Hameed S., Afzal S., Fatima S., Shakoor M. B., Bharwana S. A. and Tauqeer H. M. (2013): Heavy Metals Contamination and what are the Impacts on Living Organisms. *Greener Journal of Environmental Management and Public Safety*. ISSN: 2354-2276 Vol. 2 (4), pp. 172-179.

Sattar, M. U., Khan, M. A., Khalil, A. A. and Amir, R. M. (2013): Mitigation of Heavy Metals in Vegetables through Washing with House Hold Chemicals, 3(5), 1–12.

Schachtman, D. P., Reid, R. J. and Ayling, S. M. (1998): Update on Phosphorus Uptake by Plants: From Soil to Cell, 447–453. http://doi.org/10.1104/pp.116.2.447.

Schnug, E. and Haneklaus, H. S. (2016): The Enigma of Fertilizer Phosphorus Utilization, 1–926. http://doi.org/10.1007/978-94-017-7612-7.

Schönfeldt, H. C. and Pretorius, B. (2011): The nutrient content of five traditional South African dark green leafy vegetables-A preliminary study. Journal of Food Composition and Analysis, 24(8), 1141–1146. http://doi.org/10.1016/j.jfca.2011.04.004.

Sekara, A., Poniedziaek, M., Ciura, J. and Jedrszczyk, E. (2005): Zinc and copper accumulation and distribution in the tissues of nine crops: Implications for phytoremediation. Polish Journal of Environmental Studies, 14(6), 829–835.

Shackleton, C. M., Pasquini, M. W. and Drescher, A. W. (2009): African indigenous vegetables in urban agriculture. Earthscan. London, England.

Sharma, R. K, Agrawal, M. and Marshall, F. (2007): Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. Ecotoxicology and Environmental Safety, 66(2), 258–266. http://doi.org/10.1016/j.ecoenv.2005.11.007.

Sharma, R. K., Agrawal, M. and Marshall, F. M. (2008a): Atmospheric deposition of heavy metals (Cu, Zn, Cd and Pb) in Varanasi City, India. Environmental Monitoring and Assessment, 142(1–3), 269–278. http://doi.org/10.1007/s10661-007-9924-7.

Sharma, R. K., Agrawal, M. and Marshall, F. M. (2008b): Heavy metal (Cu, Zn, Cd and Pb) contamination of vegetables in urban India: A case study in Varanasi. Environmental Pollution, 154(2), 254–263. http://doi.org/10.1016/j.envpol.2007.10.010.

Sharma, R. K., Agrawal, M. and Marshall, F. M. (2009): Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. Food and Chemical Toxicology, 47(3), 583–591. http://doi.org/10.1016/j.fct.2008.12.016.

Shen, J., Lixing, Y., Junling, Zhang. Haigang, L., Zhaohai, B., Xinping, Chen. Weifeng, Z. and Fusuo, Z. (2011): Phosphorus Dynamics: From Soil to Plant. Plant Physiology, 156(3), 997–1005. http://doi.org/10.1104/pp.111.175232.

Shiundu, K. M. and Oniang'o, R. (2007): Marketing African leafy vegetables, challenges and opportunities in the Kenyan context. African Journal of Food Agriculture Nutrition and Development, 17: 4-12.

Shujie, M. and Yunfa, Q. (2011): Effects of phosphorus concentration on adaptive mechanisms of high-and low-P efficiency soybean genotypes when grown in solution. Plant, Soil and Environment-UZEI, 57(1998), 61–66.

Siegfried, K., Dietz, H., Schlecht, E. and Buerkert, A. (2011): Nutrient and carbon balances in organic vegetable production on an irrigated, sandy soil in northern Oman. Journal of Plant Nutrition and Soil Science, 174(4), 678–689. http://doi.org/10.1002/jpln.201000318.

Simpson, R. J., Oberson, A., Culvenor, R. A., Ryan, M. H., Veneklaas, E. J., Lambers, H.and Richardson, A. E. (2011): Strategies and agronomic interventions to improve the phosphorus-use efficiency of farming systems. Plant and Soil (Vol. 349). http://doi.org/10.1007/s11104-011-0880-1.

Singh, M. (2004): An evaluation of heavy metals concentration in vegetables grown in the vicinity of industrial area. *Journal of Agroalimentary Processes and Technology,* 17(1), 36-41.

Smaling, E. M. A., Stoorvogel, J. J. and Windmeijer, P. N. (1993): Calculating soil nutrient balances in Africa at different scales - II. District scale. Fertilizer Research, 35(3), 237–250. http://doi.org/10.1007/BF00750642.

Smaling, E. M. A., Fresco, L. O. and De Jager, A. (1996): Classifying and monitoring soil nutrient stocks and flows in African agriculture. Ambio 25, 492–496.

Smaling, E. M. A., Nandwa, S. M. and Janssen, B. H. (1997): Soil fertility in Africa is at stake. In (R.J. Buresh et al., Eds) Replenishing soil fertility in Africa. SSSA Special Publication 51. Soil Science Society of America, Madison, USA. pp. 47-61.

Smith, I. F. and Eyzaguirre, P. (2007): African leafy vegetables: Their role in the World Health Organization's Global Fruit and Vegetable Initiative. African Journal of Food Agriculture Nutrition and Development 7(3), 1–17.

Smolders, E. (2001): Cadmium uptake by plants. International Journal of Occupational Medicine and Environmental Health, 14(2), 177–183.

Soetan, K. O., Olaiya, C. O. and Oyewole, O. E. (2010): The importance of mineral elements for humans, domestic animals and plants : A review. African Journal of Food Science, 4(May), 200–222. http://doi.org/10.1186/s12302-017-0116-y.

Stein, A. J. (2010): Global impacts of human mineral malnutrition. Plant and Soil, 335(1), 133–154. http://doi.org/10.1007/s11104-009-0228-2.

Stoorvogel, J. J., Smaling E. M. A. and Janssen B. H. (1993): Calculating soil nutrient balances in Africa at different scales. Fertilizer Research. No. 35: 227-335.

Stoorvogel, J. J. and Smaling, E. M. A. (1990): Assessment of soil nutrient depletion in sub-Saharan Africa: 1983-2000. Report 28. Wageningen, The Netherlands, Winand Staring Centre.

Stylianou, M. (2007): Effect of acid treatment on the removal of heavy metals from sewage sludge. Desalination, 248(November 2006), 123–130. http://doi.org/10.1016/j.desal.0000.00.000.

Swaminathan, M.S. (2003): Biodiversity: an effective safety net against environmental pollution. Environmental Pollution 126, 287–291.

Szabela, A. D., Markiewicz J. and Wolf M. W. (2015): Heavy Metal Uptake by Herbs. IV. Influence of Soil pH on the Content of Heavy Metals in *Valeriana officinalis* L. Water Air Soil Pollut. 2015; 226(4): 106. doi: 10.1007/s11270-015-2360-3.

Taiwo, I. E., Henry, A. N. and Imbufe, A. P. (2014): Heavy metal bioaccumulation and biomarkers of oxidative stress in the wild African tiger frog, Hoplobatrachus occipitalis. African Journal of Environmental Science and Technology, 8(January), 6–15. http://doi.org/10.5897/AJEST2013.

Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N. and Mukhlisin, M. (2011): A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. International Journal of Chemical Engineering, 2011. http://doi.org/10.1155/2011/939161.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K. and Sutton, J. D. (2012): Heavy Metals Toxicity and the Environment. 101: 133–164. doi:10.1007/978-3-7643-8340-4_6.

Tittonell, P., Vanlauwe, B., Corbeels, M. and Giller, K. E. (2008): Yield gaps, nutrient use efficiencies and response to fertilisers by maize across heterogeneous smallholder farms of western Kenya. Plant and Soil, 313(1–2), 19–37. http://doi.org/10.1007/s11104-008-9676-3.

UN (2014). World Urbanization Prospects. The 2014 Revision. ISBN 978-92-1-151517-6.

Uraguchi, S., Mori, S., Kuramata, M., Kawasaki, A., Arao, T. and Ishikawa, S. (2009): Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. Journal of Experimental Botany, 60(9), 2677–2688. http://doi.org/10.1093/jxb/erp119.

Uusiku, N. P., Oelofse, A., Duodu, K. G., Bester, M. J. and Faber, M. (2010): Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. Journal of Food Composition and Analysis, 23(6), 499–509. http://doi.org/10.1016/j.jfca.2010.05.002.

Van den Bosch, H., De Jager, A. and Vlaming, J. (1998): Monitoring nutrient flows and economic performance in African farming systems (NUTMON).

Van der Hoeven, M., Faber, M., Osei, J., Kruger, A. and Smuts, C. M. (2016): Effect of African leafy vegetables on the micronutrient status of mildly deficient farm-school children in South Africa: a randomized controlled study. Public Health Nutrition, 19(5), 935–945. http://doi.org/10.1017/S1368980015002037.

Van Jaarsveld, P., Faber, M., van Heerden, I., Wenhold, F., Jansen van Rensburg, W. and Van Averbeke, W. (2014): Nutrient content of eight African leafy vegetables and their potential contribution to dietary reference intakes. Journal of Food Composition and Analysis, 33(1), 77–84. http://doi.org/10.1016/j.jfca.2013.11.003.

Vance, C., Uhde-Stone, C. and Allan, D. (2003): Phosphorus aquisition and use: critical adaptations by plants for securing a nonrewable resource. New Phytologist, 157, 423–447. http://doi.org/10.1046/j.1469-8137.2003.00695.x.

Vanlauwe, B., Descheemaeker, K., Giller, K. E., Huising, J., Merckx, R., Nziguheba, G. and Zingore, S. (2015): Integrated soil fertility management in sub-Saharan Africa: unravelling local adaptation. Soil, 1(1), 491–508. http://doi.org/10.5194/soil-1-491-2015.

Vanlauwe, B., Wendt, J., Giller, K. E., Corbeels, M., Gerard, B. and Nolte, C. (2014): A fourth principle is required to define Conservation Agriculture in sub-Saharan Africa: The appropriate use of fertilizer to enhance crop productivity. Field Crops Research, 155, 10–13. http://doi.org/10.1016/j.fcr.2013.10.002.

Vejchasarn, P., Lynch, J. P. and Brown, K. M. (2016): Genetic Variability in Phosphorus Responses of Rice Root Phenotypes. Rice, 9(1), 29. http://doi.org/10.1186/s12284-016-0102-9.

Veneklaas, E. J., Lambers, H., Bragg, J., Finnegan, P. M., Lovelock, C. E., Plaxton, W. C., Price, C. A., Scheible, W. R., Shane, M. W., White, P. J. and Raven, J. A. (2012): Opportunities for improving phosphorus- use efficiency in crop plants. New Phytol 195:306–320.

Waddell, H. A., Simpson, R. J., Ryan, M. H., Lambers, H., Garden, D. L. and Richardson, A. E. (2017): Root morphology and its contribution to a large root system for phosphorus uptake by Rytidosperma species (wallaby grass). Plant and Soil, 412(1–2), 7–19. http://doi.org/10.1007/s11104-016-2933-y.

Wang, X., Shen, J. and Liao, H. (2010): Acquisition or utilization, which is more critical for enhancing phosphorus efficiency in modern crops? Plant Science, 179(4), 302–306. http://doi.org/10.1016/j.plantsci.2010.06.007.

Wang, Y., Zhang, H., Tang, J., Xu, J., Kou, T. and Huang, H. (2015). Accelerated phosphorus accumulation and acidification of soils under plastic greenhouse condition in four representative organic vegetable cultivation sites. Scientia Horticulturae, 195(M), 67–73. http://doi.org/10.1016/j.scienta.2015.08.041.

Wehmeyer, A. S. (1986): Edible Wild Plants of South- ern Africa: Data on the Nutrient Contents of over 300 Species. CSIR, Pretoria.

Weinberger K. and Msuya J. (2004): Indigenous Vegetables in Tanzania- Significance and Prospects. Shanhua, Taiwan: AVRDC-The World Vegetable Center, Technical Bulletin No. 31, AVRDC Publication 04-600, 70 p.

Weldegebriel, Y., Chandravanshi, B. S. and Wondimu, T. (2012): Concentration levels of metals in vegetables grown in soils irrigated with river water in Addis Ababa, Ethiopia. Ecotoxicology and Environmental Safety, 77, 57–63. http://doi.org/10.1016/j.ecoenv.2011.10.011.

White J. P (2012): Heavy metal toxicity in plants. Scottish Crop Research Institute, Invergowrie, UK, 210 – 237.

WHO/FAO (2006). Guidelines on food fortification with micronutrients. Unscn.Org, 341. http://doi.org/10.1242/jeb.02490.

WHO (1995): Environmental Health Criteria, vol. 165. Inorganic lead. International Programme on Chemical Safety, Geneva.

WHO (2007): Health risks of heavy metals from long-range transboundary air poluution. Joint WHO L Convention Task Force on Health Aspects of Air Pollution, 2–144. http://doi.org/10.1002/em.

WHO (2010): Exposure to lead: A major public health concern

Williamson, L. C., Ribrioux, S., Fitter, A. H. and Leyser, H. M. O. (2001): Phosphate availability regulates root system architecture in Arabidopsis. Plant Physiology, 126(2), 875–882. http://doi.org/10.1104/pp.126.2.875.

Wong, C. S. C., Li, X. D., Zhang, G., Qi, S. H. and Peng, X. Z. (2003): Atmospheric depositions of heavy metals in the Pearl River Delta, China. Atmospheric Environment 37, 767–776.

World Bank (1998): Soil Fertility Manangement in Sub − Saharan Africa, 58.

Wuana, R. A. and Okieimen, F. E. (2011): Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. ISRN Ecology, 2011, 1–20. http://doi.org/10.5402/2011/402647.

Yahaya, Y., Bagudo, B. U. and Noma, S. S. (2011): Seasonal variation in nutrient content of some selected vegetables from Wamakko, Sokoto State, Nigeria, 2(April), 117–125.

Yevich, R. and Logan, A. J. (2003): An assessment of biofuel use and burning of agricultural waste in the developing world, Global Biogeochem. Cycles, 17(4), 1095, doi: 10.1029/2002GB001952, 2003.

Zhu, Y. and Smith, S. E. (2001): Seed phosphorus (P) content affects growth, and P uptake of wheat plants and their association with arbuscular mycorrhizal (AM) fungi, 105–112.

Ziblim, A. I., Khan A. T. and Akaffo P. (2012): Effects of Season on the Mineral (Potassium, Calcium, Phosphorus, Magnesium) Levels of Pennisetum Pedicellatum in Northern Ghana. Greener Journal of Agricultural Sciences ISSN: 2276-7770 Vol. 2 (7), pp. 329-333.

Zingore, S., Murwira, H. K., Delve, R. J. and Giller, K. E. (2007). Influence of nutrient management strategies on variability of soil fertility, crop yields and nutrient balances on smallholder farms in Zimbabwe. Agriculture, Ecosystems and Environment, 119(1–2), 112–126. http://doi.org/10.1016/j.agee.2006.06.019.

Declaration

I hereby declare that I completed the doctoral thesis independently based on the stated resources and aids. I have not applied for a doctoral degree elsewhere and do not have a corresponding doctoral degree. I have not submitted the doctoral thesis, or parts of it, to another academic institution and the thesis has not been accepted or rejected. I declare that I have acknowledged the Doctoral Degree Regulations, which underlie the procedure of the Faculty of Life Sciences of Humboldt-Universität zu Berlin, as amended on 5th March 2015. Furthermore, I declare that no collaboration with commercial doctoral degree supervisors took place, and that the principles of Humboldt-Universität zu Berlin for ensuring good academic practice were abided by.

Berlin, August, 2018.